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(54) Title: METHODS OF TREATING NEUROINFLAMMATORY CONDITIONS

(57) Abstract: Provided herein are compounds and compositions thereof for modulating hepatocyte growth factors. In some embodiments, the compounds and compositions are provided for treatment of diseases, including neuroinflammatory diseases.

METHODS OF TREATING NEUROINFLAMMATORY CONDITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of US Provisional Application No. 63/338,328, filed May 4, 2022; US Provisional Application No. 63/414,111, filed October 7, 2022; US Provisional Application No. 63/424,352, filed November 10, 2022; and US Provisional Application No. 63/447,432, filed February 22, 2023, each of which is incorporated by reference herein in its entirety for any purpose.

FIELD

[0002] The present disclosure relates generally to compounds, compositions, and methods for treating diseases, such as neuroinflammatory conditions, such as multiple sclerosis, stroke, a frontotemporal dementia, an encephalopathy, or an encephalitis.

BACKGROUND

[0003] Hepatocyte growth factor (HGF) is a pleiotropic protein factor involved in numerous biological processes including embryonic and organ development, regeneration, and inflammation. HGF is a critical contributor to cortical, motor, sensory, sympathetic, and parasympathetic neuronal development and maturation. HGF is translated and secreted as inactive pro-HGF, but following cleavage, the resultant α and β-subunits are joined by a disulfide linkage to form the active heterodimer. Expression of HGF predominantly occurs in mesenchymal cells such as fibroblasts, chondroblasts, adipocytes, and the endothelium. Expression has also been demonstrated in the central nervous system (CNS) including neurons, astrocytes, and ependymal cells (Nakamura and Mizuno, 2010).

[0004] All biological activities of HGF are mediated through MET, a transmembrane receptor tyrosine kinase that serves as the sole known receptor for HGF. MET has known involvement in a variety of biological processes, with demonstrated roles in development, regeneration, and response to injury. Upon binding of HGF to the extracellular domain of MET, homo-dimerization of the MET protein leads to auto-phosphorylation of the intracellular domain. Phosphorylation of MET intracellular domains leads to recruitment and phosphorylation of a variety of effector proteins including Gab1, GRB2, Phospholipase C, and Stat3 (Gherardi et al., 2012; Organ and Tsao, 2011). These effector proteins then interact with downstream signaling pathways including PI3K/Akt, Ras/Raf/MAPK, RAC1/CDC42, and RAP/FAK among others to influence an array of cellular components including gene regulation, cytoskeletal rearrangements, cell cycle progression, cell adhesion, survival, and proliferation (Organ and Tsao, 2011).

[0005] Several lines of evidence indicate that HGF/MET may be a viable target for neuroinflammatory conditions. HGF is a critical regulator of inflammation and autoimmunity. HGF activity reduces the expression of the pro-inflammatory cytokine IL-6 and promotes expression of the anti-inflammatory Il-10 in monocytes (Molnarfi et al., 2015). Additionally, HGF increases tolerogenic dendritic cells and attenuates cytotoxic T-cell activity (Ilangumaran et al., 2016). These effects of HGF activity on inflammation and immune function would likely be beneficial in neuroinflammatory conditions.

[0006] For multiple sclerosis therapy, levels of HGF in cerebrospinal fluid (CSF) are negatively correlated with disease activity in MS patients. HGF levels are reduced when the disease is in a flare-up phase, but HGF levels rise again as the disease enters a remission phase (Muller et al., 2012). It is not yet clear whether the reduction in CSF HGF levels are causative of the increased rate of disease presentation, but the regenerative properties of HGF on oligodendrocytes (Yan and Rivkees, 2002) may be keeping the disease in check in the short term. Some clinical trials reported improvement in MS patients following transplantation of mesenchymal stem cell (MSC) progenitor cells (Harris et al., 2018), and this improvement is likely attributed to the high levels of HGF secreted by MSCs (Bai Lianhua et al., 2012).

[0007] Stroke occurs when the flow of blood to brain structures is interrupted. Stroke can generally be cause by ischemia or hemorrhage. Ischemic stroke is a disease involving reduced blood flow to the brain, or certain areas as caused by occlusion of blood vessels. This can lead to a cascade of responses including lack of oxygen, insufficient energy availability, excess excitatory signaling leading to excess calcium, which ultimately results in excitotoxicity and release of reactive oxygen species. Initiation of these maladaptive signaling pathways results in glial scar formation and/or cell death via apoptosis (Campbell et al., 2019). Ischemic events can also be triggered subsequent to subarachnoidal hemorrhage, caused by aneurysm, and extravasation of factors leading to vessel occlusion.

[0008] Most treatments for stroke are either focused on preventing stroke recurrence or removal of the blood clot via thrombectomy or thrombolysis (Campbell et al., 2019). The latter treatments can be quite expensive and invasive, as they require a surgical procedure. Other innovative treatments, such as modulation of HGF signaling, were reported to promote stroke recovery through the involvement neurotrophic and regenerative mechanisms instead of surgical intervention. Studies have shown that continuous infusion of HGF can promote tissue preservation in the acute stroke phase (Date et al., 2006). Likewise, acute HGF delivery in the first three days after stroke can promote neuroprotection along with motor coordination recovery and reduce infarct volume (Doeppner Thorsten R et al., 2011). Therefore, because of its

neurotrophic, anti-apoptotic, and regenerative features, HGF modulation could be a promising therapeutic option in stroke recovery.

[0009] Hemorrhagic stroke occurs secondary to hematoma formation in the brain. Thrombin is released to induce clotting, which slows the hemorrhage and initiates a cascade that ultimately results in edema, apoptosis of neurons and glia, excitotoxicity, microglial activation, and release of inflammatory mediators including but not limited to, TNF-α, IL-6, and matrix metalloproteinase-9 (Unnithan and Mehta, 2022; Chen et al., 2014).

[0010] Treatments for hemorrhagic stroke center primarily around the reduction of ongoing hemorrhage and preventing further damage by secondary effects including seizure and increased intracranial pressure. Neuroprotective treatments targeting inflammation, excitotoxicity and oxidative stress are also being evaluated (Unnithan and Mehta, 2022). Treatments involving the modulation of the neuroprotective HGF system are well aligned to reduce the impact of these sources of neuroinjury (Desole et al., 2021). Studies evaluating the transplant of immortalized neuronal stem cells into an animal model of hemorrhagic stroke showed improvement in behavioral outcomes compared to control animals. This improvement was linked in part to the increased expression of HGF by the implanted stem cells (Hong et al., 2007), indicating that modulation of HGF may be a viable neuroprotective treatment for hemorrhagic stroke.

[0011] Frontotemporal dementia (FTD) is the second most common cause of dementia and is characterized by various degrees of language dysfunction and behavior disorders. Like many other neurological and neurodegenerative diseases, FTD disease progression and symptom presentation may be driven by neuroinflammatory processes. Modulation of HGF/MET signaling is involved in the development of FTD, as studies of neuroinflammatory signatures in FTD patients identify dysregulation of HGF expression (Boström et al., 2021). Small-molecule modulation of HGF/MET signaling could therefore be expected to limit the progression of FTD symptoms in human patients.

[0012] Encephalopathies of idiopathic origin may also be treated by modulation of HGF/MET signaling. Alteration of brain function in encephalopathic patients can result from a number of sources, many of which may respond to HGF/MET signaling by suppressing apoptotic neuron death and reducing neuroinflammation. Septic encephalopathy is brain dysfunction mediated by septic inflammatory response independent of other organ dysfunction, and HGF expression profiles are altered in patients with sepsis that result in long-term cognitive impairment (Andonegui et al., 2018), indicating that HGF/MET signaling is being activated as an endogenous response to neurologically relevant sepsis. Other forms of encephalopathy that may respond to HGF/MET signaling modulation include, but are not limited to, hepatic

encephalopathy, metabolic encephalopathy, hypoxic encephalopathy, Hashimoto encephalopathy, and chronic traumatic encephalopathy.

[0013] Encephalitis refers to inflammation of the brain that may result in brain swelling fever, headache, confusion, and seizures. The source of the inflammation can be widely varied, including due to viral, bacterial, parasitic, or fungal infection or due to autoimmune diseases.

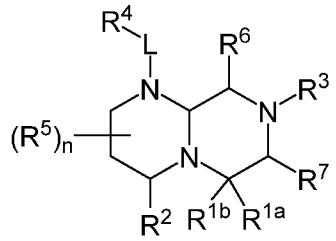
[0014] Treatment generally depends on the cause of the encephalitis (i.e. anti-viral medications to treat viral encephalitis, antibiotics to treat bacterial encephalitis). However, treatments that address the underlying inflammation could also be helpful in encephalitic diseases. The HGF/MET signaling pathway has been reported to be a potent immunomodulatory system in various disease models with inflammatory influences (Gong et al., 2006; Mizuno and Nakamura, 2012; Mizuno et al., 1998; Vallarola et al., 2020) and is also known to inhibit apoptosis (Xiao et al., 2001), which is a contributing factor in most types of encephalitis.

[0015] Although progress has been made in this field, there remains a need for improved compounds and methods for treatment of neuroinflammatory conditions.

SUMMARY

[0016] Provided herein are compounds that modulate HGF for use in treating neuroinflammatory conditions, such as multiple sclerosis, stroke, a frontotemporal dementia, an encephalopathy, or an encephalitis. Nonlimiting exemplary embodiments include:

1. A method of treating a neuroinflammatory condition in a subject in need thereof, comprising administering an effective amount of a compound of Formula (I):



(I)

or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, wherein:

L is a direct bond, -C(=O)-, -$(CR^aR^b)_m$-C(=O)-, -C(=O)-$(CR^aR^b)_m$-, or -$(CR^aR^b)_m$-;

each R^a and R^b is independently H, C_1-C_6 alkyl, C_2-C_6 alkenyl, or C_2-C_6 alkynyl;

R^{1a} and R^{1b} are independently H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, halo, or C_6-C_{10} arylalkyl;

R^2 is H, oxo, or thioxo;

R^3 is C_2-C_6 alkyl, C_3-C_6 alkenyl, C_3-C_6 alkynyl, C_3-C_{12} cycloalkyl, C_3-C_6 cycloalkylalkyl, C_6-C_{10} arylalkyl, 5- to 10-membered heteroarylalkyl, or 5- to 10-membered heterocyclylalkyl,

wherein the 5- to 10-membered heteroarylalkyl or 5- to 10-membered heterocyclylalkyl contains 1-3 heteroatoms selected from nitrogen and oxygen;

R^4 is C_6-C_{10} aryl, 5- to 10-membered heteroaryl, or 5- to 10-membered heterocyclyl,

wherein the 5- to 10-membered heteroaryl or 5- to 10-membered heterocyclyl contains 1-3 heteroatoms selected from nitrogen and oxygen;

each R^5 is independently C_1-C_6 alkyl, oxo, or halo;

R^6 is H, C_1-C_6 alkyl, or oxo;

R^7 is H or oxo;

m is 1 or 2; and

n is an integer from 0 to 3;

wherein each C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{12} cycloalkyl, C_3-C_{12} cycloalkylalkyl, C_6-C_{10} aryl, C_6-C_{10} arylalkyl, 5- to 10-membered heteroaryl, 5- to 10-membered heteroarylalkyl, 5- to 10-membered heterocyclyl, and 5- to 10-membered heterocyclylalkyl is optionally substituted with one to five substituents selected from hydroxyl, halo, amino, C_1-C_6 haloalkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkoxy, cyano, -(C=O)NH_2, nitro, -SO_2(C_1-C_6 alkyl), and -CO_2H.

2. The method of embodiment 1, wherein L is -C(=O)- or -(CR^aR^b)$_m$-.

3. The method of embodiment 1 or 2, wherein L is a -C(=O)-.

4. The method of embodiment 1 or 2, wherein L is -(CR^aR^b)$_m$-.

5. The method of embodiment 4, wherein R^a and R^b are each H, and m is 1.

6. The method of any one of embodiments 1-5, wherein R^{1a} and R^{1b} are each independently H; C_1-C_6 alkyl optionally substituted with 1-3 substituents selected from halo, -CO_2H, and -C(=O)NH_2; C_1-C_6 alkoxy; halo; or C_6-C_{10} arylalkyl optionally substituted by 1-3 substituents selected from halo and amino.

7. The method of embodiment 6, wherein R^{1a} and R^{1b} are each independently H, methyl, fluoro, 2-methylbutyl, -CH_2F, methoxy, -CH_2CO_2H, -CH_2C(=O)NH_2, benzyl, or 4-aminobenzyl.

8. The method of embodiment 6, wherein R^{1a} and R^{1b} are each independently H or C_1-C_3 alkyl.

9. The method of embodiment 8, wherein R^{1a} is methyl and R^{1b} is H.

10. The method of embodiment 8, wherein R^{1a} and R^{1b} are each H.

11. The method of any one of embodiments 1-10, wherein R^2 is H.

12. The method of any one of embodiments 1-10, wherein R^2 is thioxo.

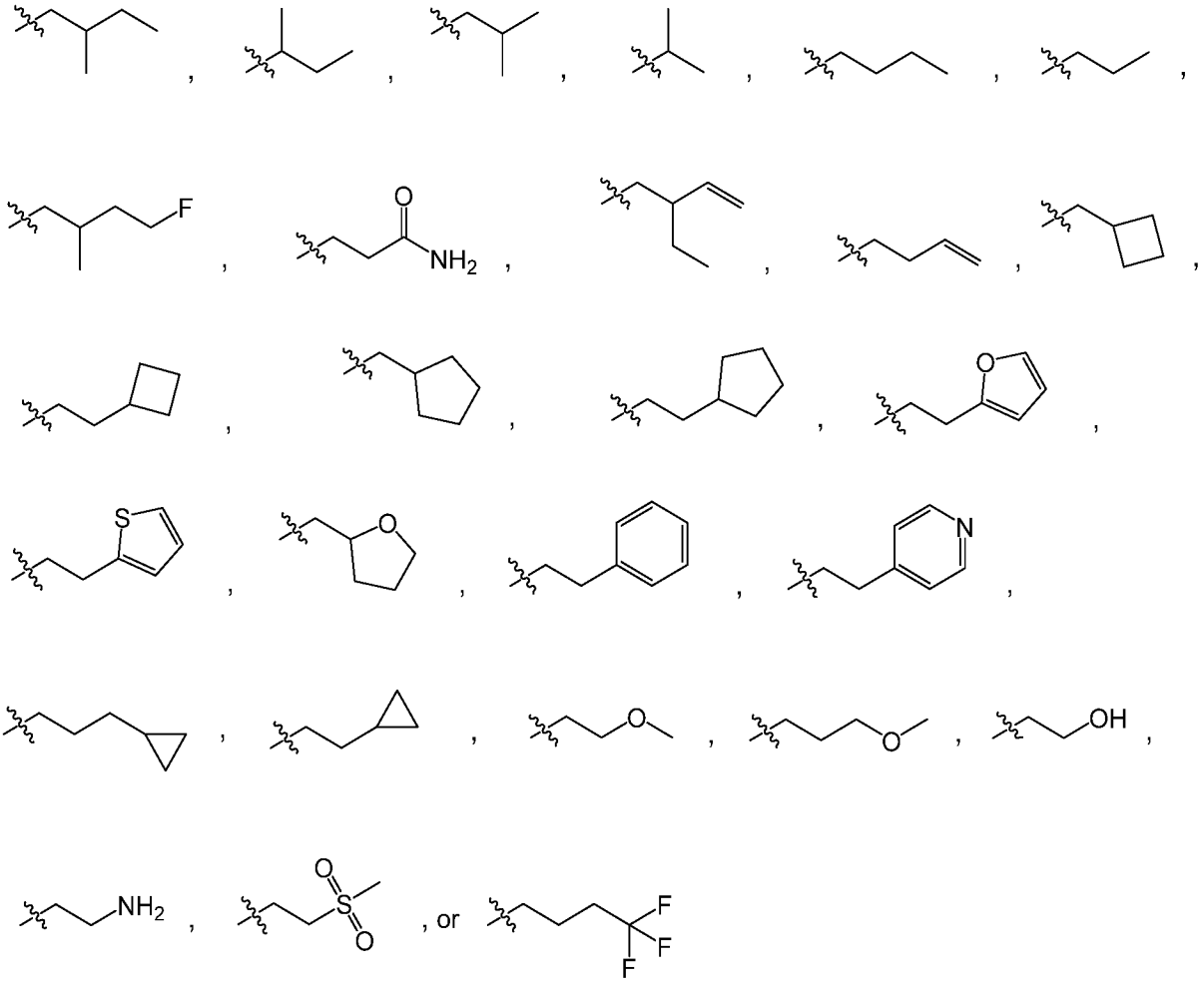
13. The method of any one of embodiments 1-10, wherein R^2 is oxo.

14. The method of any one of embodiments 1-13, wherein R^3 is C_3-C_6 alkyl, C_3-C_6 alkenyl, C_3-C_6 alkynyl, C_3-C_{12} cycloalkyl, C_3-C_6 cycloalkylalkyl, C_6-C_{10} arylalkyl, 5- to 10-membered heteroarylalkyl, or 5- to 10-membered heterocyclylalkyl, wherein the alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, arylalkyl, heteroarylalkyl, or heterocyclylalkyl is optionally substituted with one to five substituents selected from hydroxyl, halo, amino, C_1-C_6 haloalkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkoxy, cyano, -(C=O)NH_2, nitro, -SO_2(C_1-C_6 alkyl), and -CO_2H.

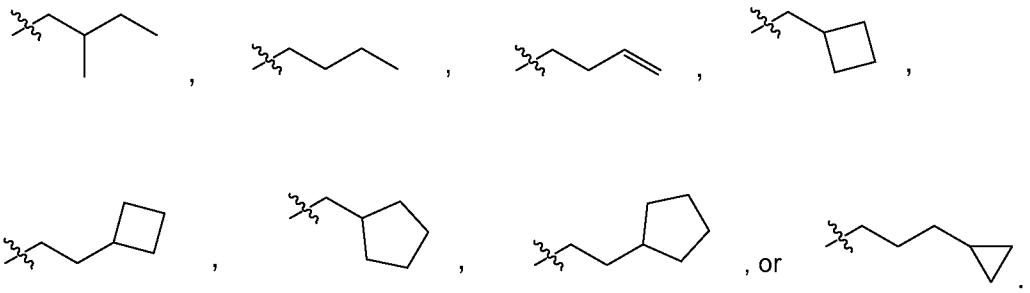
15. The method of any one of embodiments 1-13, wherein R^3 is C_2-C_6 alkyl optionally substituted by 1-3 substituents selected from halo, C_1-C_3 alkoxy, hydroxy, -NH_2, -SO_2(C_1-C_3 alkyl), and -C(=O)NH_2; C_2-C_6 alkenyl; C_3-C_6 cycloalkylalkyl; 5- to 6-membered heteroarylalkyl; 5- to 6-membered heterocyclylalkyl; or C_6 arylalkyl.

16. The method of embodiment 15, wherein R^3 is C_2 alkyl substituted by 1-3 substituents selected from C_1-C_3 alkoxy, hydroxy, -NH_2, and -SO_2(C_1-C_3 alkyl).

17. The method of any one of embodiments 14-16, wherein R^3 is:



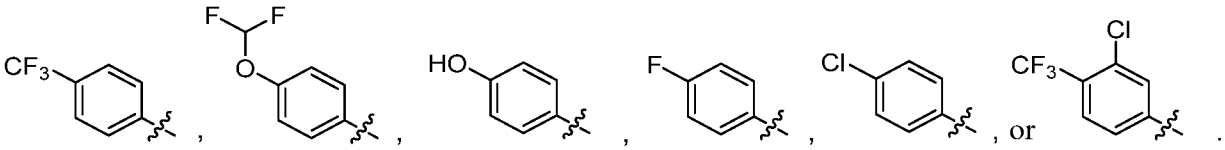
18. The method of embodiment 17, wherein R^3 is:



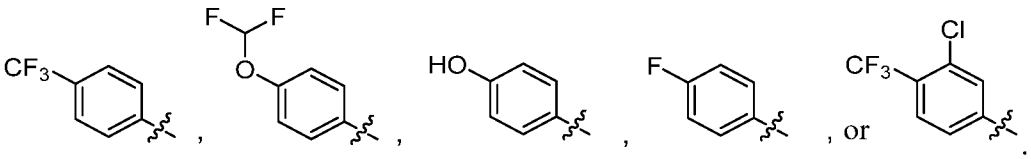
19. The method of any one of embodiments 1-18, wherein R^4 is C_6-C_{10} aryl optionally substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy.

20. The method of embodiment 19, wherein R^4 is phenyl substituted with 1-3 substituents selected from $-CF_3$, $-OCHF_2$, -OH, fluoro, and chloro.

21. The method of embodiment 20, wherein R^4 is:



22. The method of embodiment 21, wherein R^4 is:

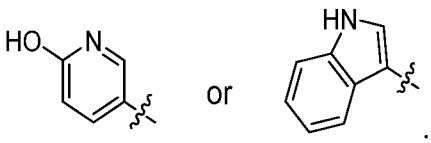


23. The method of any one of embodiments 1-18, wherein R^4 is 5- to 10-membered heteroaryl optionally substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy.

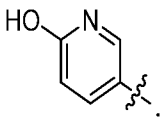
24. The method of embodiment 23, wherein

R^4 is pyridyl or indolyl optionally substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy.

25. The method of embodiment 24, wherein

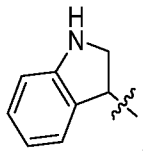
R^4 is .

26. The method of embodiment 25, wherein

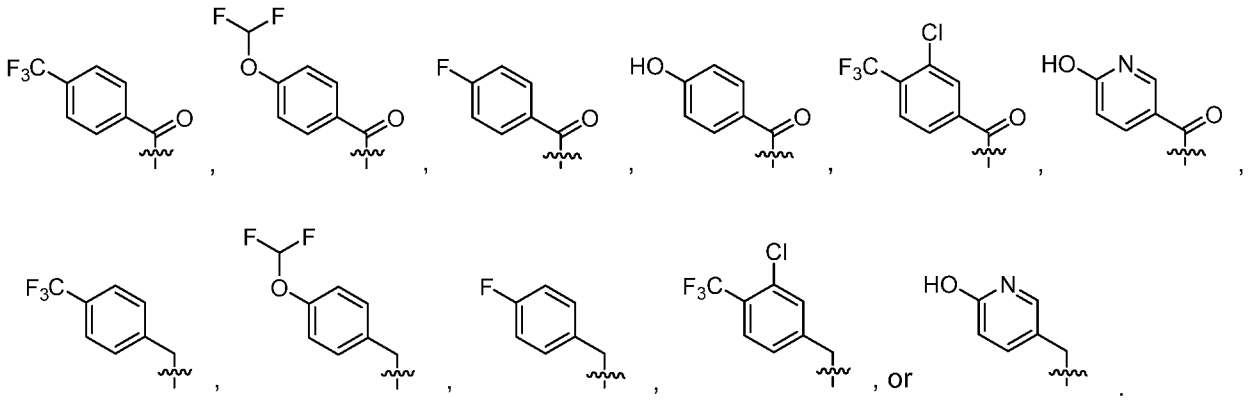
R^4 is .

27. The method of any one of embodiments 1-18, wherein R^4 is 5- to 10-membered heterocyclyl optionally substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy.

28. The method of embodiment 27, wherein R^4 is indolinyl.

29. The method of embodiment 28, wherein R^4 is .

30. The method of any one of embodiments 1-26, wherein -L-R^4 is:



31. The method of any one of embodiments 1-30, wherein n is 0.

32. The method of any one of embodiments 1-30, wherein n is 1.

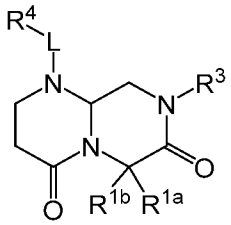
33. The method of embodiment 32, wherein R^5 is oxo or halo.

34. The method of embodiment 33, wherein R^5 is oxo or fluoro.

35. The method of any one of embodiments 1-34, wherein R^6 is H.

36. The method of any one of embodiments 1-35, wherein R^7 is oxo.

37. The method of any one of embodiments 1-10, 13-31, 35, and 36, wherein the compound is of Formula (V):



(V) , or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof.

38. The method of embodiment 37, wherein:

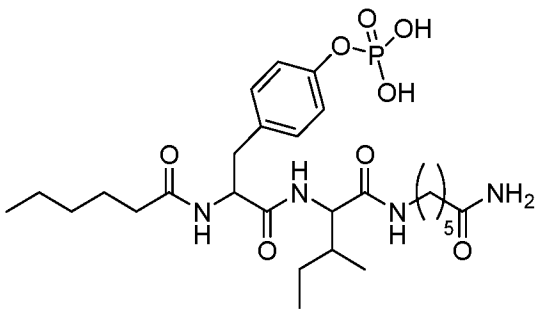
L is -C(=O)- or -CH_2-;

R^{1a} and R^{1b} are independently H or C_1-C_3 alkyl optionally substituted with -CO_2H;

R^3 is C_4-C_5 alkyl, C_4-C_5 alkenyl, or C_1-C_3 alkyl substituted with C_3-C_5 cycloalkyl; and

R^4 is phenyl or pyridyl substituted with 1-3 substituents selected from -CF_3, -$OCHF_2$, -OH, fluoro, and chloro.

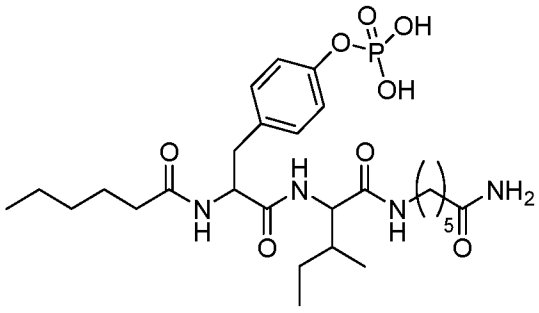
39. A method of treating a neuroinflammatory condition in a subject in need thereof, comprising administering an effective amount of compound A19:



A19

or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof.

40. A method of treating a neuroinflammatory condition in a subject in need thereof, comprising administering an effective amount of a compound selected from the compounds of Table 1A and compound A19:



A19

and pharmaceutically acceptable salts, isotopic forms, and stereoisomers thereof.

41. The method of any one of the preceding embodiments, wherein the neuroinflammatory condition is multiple sclerosis, stroke, a frontotemporal dementia, an encephalopathy, or an encephalitis.

42. The method of any one of embodiments 1-41, wherein the neuroinflammatory condition is multiple sclerosis.

43. The method of any one of embodiments 1-41, wherein the neuroinflammatory condition is a stroke.

44. The method of embodiment 43, wherein the neuroinflammatory condition is an ischemic stroke.

45. The method of embodiment 43, wherein the neuroinflammatory condition is a hemorrhagic stroke.

46. The method of any one of embodiments 1-41, wherein the neuroinflammatory condition is a frontotemporal dementia.

47. The method of embodiment 46, wherein the frontotemporal dementia is idiopathic.

48. The method of embodiment 46, wherein the frontotemporal dementia is a result of progranulin mutation associated linked to chromosome 17 (p17).

49. The method of any one of embodiments 1-41, wherein the neuroinflammatory condition is an encephalopathy.

50. The method of embodiment 49, wherein the encephalopathy is associated with hypoxia, such as small vessel encephalopathy ("Binswanger's disease"), multi-infarct dementia, asphyxia, or ischemia.

51. The method of embodiment 49, wherein the encephalopathy is chronic traumatic encephalopathy.

52. The method of any one of embodiments 1-41, wherein the neuroinflammatory condition is an encephalitis.

53. The method of embodiment 52, wherein the encephalitis is an autoimmune encephalitis, viral encephalitis or bacterial encephalitis.

54. The method of embodiment 53, wherein the autoimmune encephalitis is N-methyl D-aspartate receptor (NMDAR)-encephalitis.

55. The method of any one of embodiments 1-54, wherein the compound reduces inflammation associated with the inflammatory condition.

56. The method of any one of embodiments 1-55, wherein the compound reduces hypoxia associated with the inflammation.

57. The method of any one of embodiments 1-56, wherein the compound improves neuronal survival and/or suppresses apoptotic cell death resulting from inflammation.

58. The method of any one of the preceding embodiments, wherein the compound is formulated as a pharmaceutical composition.

59. The method of any one of embodiments 1-58, wherein the neuroinflammatory condition is not caused by peripheral inflammation or a disease or disorder of the peripheral nervous system.

60. The method of any one of embodiments 1-59, wherein the neuroinflammatory condition is not caused by Alzheimer's disease, mild cognitive impairment, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, spinal cord injury, traumatic brain injury, or sensorineural hearing and vision loss.

61. The method of any one of embodiments 1-60, wherein the neuroinflammatory condition is not associated with peripheral inflammation or a disease or disorder of the peripheral nervous system.

62. The method of any one of embodiments 1-61, wherein the neuroinflammatory condition is not associated with Alzheimer's disease, mild cognitive impairment, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, spinal cord injury, traumatic brain injury, or sensorineural hearing and vision loss.

63. The method of any one of embodiments 1-62, wherein the subject is not suffering from Alzheimer's disease, mild cognitive impairment, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, spinal cord injury, traumatic brain injury, sensorineural hearing and vision loss, or a disease or disorder of the peripheral nervous system.

DETAILED DESCRIPTION

Definitions

[0017] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this disclosure belongs. In the following description, certain specific details are set forth in order to provide a thorough understanding of various embodiments of the disclosure. It is to be understood that the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of any subject matter claimed. To the extent any material

incorporated herein by reference is inconsistent with the express content of this disclosure, the express content controls. In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an", and "the" include plural referents unless the context clearly dictates otherwise. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, use of the term "including" as well as other forms, such as "include", "includes," and "included," is not limiting.

[0018] Unless the context requires otherwise, throughout the present specification and claims, the word "comprise" and variations thereof, such as, "comprises" and "comprising" are to be construed in an open, inclusive sense, that is, as "including, but not limited to".

[0019] In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Also, any number range recited herein relating to any physical feature, such as polymer subunits, size, or thickness, are to be understood to include any integer within the recited range, unless otherwise indicated. As used herein, the terms "about" and "approximately" mean ± 20%, ± 10%, ± 5%, or ± 1% of the indicated range, value, or structure, unless otherwise indicated.

[0020] Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present disclosure. Thus, the appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

[0021] "Amino" refers to the $-NH_2$ radical.

[0022] "Carboxy" or "carboxyl" refers to the $-CO_2H$ radical.

[0023] "Cyano" refers to the -CN radical.

[0024] "Hydroxy" or "hydroxyl" refers to the -OH radical.

[0025] "Nitro" refers to the $-NO_2$ radical.

[0026] "Oxo" refers to the =O substituent.

[0027] "Thioxo" refers to the =S substituent.

[0028] "Thiol" refers to the -SH substituent.

[0029] "Alkyl" refers to an unbranched or branched saturated hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, having from one to twelve carbon atoms (C_1-

C_{12} alkyl), preferably one to eight carbon atoms (C_1-C_8 alkyl), one to six carbon atoms (C_1-C_6 alkyl), or one to three carbon atoms (C_1-C_3 alkyl) and which is attached to the rest of the molecule by a single bond, *e.g.*, methyl, ethyl, *n*-propyl, 1-methylethyl (*iso*-propyl), *n*-butyl, *n*-pentyl, 1,1-dimethylethyl (*t*-butyl), 3-methylhexyl, 2-methylhexyl and the like. Unless stated otherwise specifically in the specification, an alkyl group is optionally substituted.

[0030] "Alkenyl" refers to an unbranched or branched unsaturated hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, which contains one or more carbon-carbon double bonds, having from two to twelve carbon atoms (C_2-C_{12} alkenyl), preferably two to eight carbon atoms (C_2-C_8 alkenyl) or two to six carbon atoms (C_2-C_6 alkenyl), and which is attached to the rest of the molecule by a single bond, *e.g.*, ethenyl, prop-1-enyl, but-1-enyl, pent-1-enyl, penta-1,4-dienyl, and the like. Unless stated otherwise specifically in the specification, an alkenyl group is optionally substituted.

[0031] "Alkynyl" refers to an unbranched or branched unsaturated hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, which contains one or more carbon-carbon triple bonds, having from two to twelve carbon atoms (C_2-C_{12} alkynyl), preferably two to eight carbon atoms (C_2-C_8 alkynyl) or two to six carbon atoms (C_2-C_6 alkynyl), and which is attached to the rest of the molecule by a single bond, *e.g.*, ethynyl, propynyl, butynyl, pentynyl, hexynyl, and the like. Unless stated otherwise specifically in the specification, an alkynyl group is optionally substituted.

[0032] "Alkoxy" refers to a radical of the formula -OR_a where R_a is an alkyl radical as defined above containing one to twelve carbon atoms. Preferred alkoxy groups have one to six carbon atoms (*i.e.*, C_1-C_6 alkoxy) or one to three carbon atoms (*i.e.*, C_1-C_3 alkoxy) in the alkyl radical. Unless stated otherwise specifically in the specification, an alkoxy group is optionally substituted.

[0033] "Aromatic ring" refers to a cyclic planar portion of a molecule (*i.e.*, a radical) with a ring of resonance bonds that exhibits increased stability relative to other connective arrangements with the same sets of atoms. Generally, aromatic rings contain a set of covalently bound co-planar atoms and comprise a number of π-electrons (for example, alternating double and single bonds) that is even but not a multiple of 4 (*i.e.*, $4n + 2$ π-electrons, where $n = 0, 1, 2, 3$, etc.). Aromatic rings include, but are not limited to, phenyl, naphthenyl, imidazolyl, pyrrolyl, pyridinyl, pyrimidinyl, pyrazinyl, pyridonyl, pyridazinyl, pyrimidonyl. Unless stated otherwise specifically in the specification, an aromatic ring includes all radicals that are optionally substituted.

[0034] "Aryl" refers to a carbocyclic ring system radical comprising 6 to 18 carbon atoms and at least one aromatic ring (*i.e.*, C_6-C_{18} aryl), preferably having 6 to 10 carbon atoms (*i.e.*, C_6-

C_{10} aryl). For purposes of embodiments of this disclosure, the aryl radical is a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems. Aryl radicals include, but are not limited to, aryl radicals derived from aceanthrylene, acenaphthylene, acephenanthrylene, anthracene, azulene, benzene, chrysene, fluoranthene, fluorene, *as*-indacene, *s*-indacene, indane, indene, naphthalene, phenalene, phenanthrene, phenyl, pleiadene, pyrene, and triphenylene. Unless stated otherwise specifically in the specification, an aryl group is optionally substituted.

[0035] "Arylalkyl" refers to a radical of the formula $–R_b-R_c$ where R_b is an alkylene chain and R_c is one or more aryl radicals as defined above, for example, benzyl, diphenylmethyl and the like. An arylalkyl group may contain a C_1-C_{10} alkylene chain connected to a C_6-C_{10} aryl radical (*i.e.*, C_6-C_{10} arylalkyl). Unless stated otherwise specifically in the specification, an arylalkyl group is optionally substituted.

[0036] "Cycloalkyl" refers to a stable non-aromatic monocyclic or polycyclic carbocyclic radical consisting solely of carbon and hydrogen atoms, which may include fused or bridged ring systems, having from three to fifteen carbon atoms (*i.e.*, C_3-C_{15} cycloalkyl), preferably having from three to ten carbon atoms (*i.e.*, C_3-C_{10} cycloalkyl) or three to six carbon atoms (*i.e.*, C_3-C_6 cycloalkyl), and which is saturated or unsaturated and attached to the rest of the molecule by a single bond. Monocyclic radicals include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Cycloalkyl also includes "spiro cycloalkyl" when there are two positions for substitution on the same carbon atom. Polycyclic radicals include, for example, adamantyl, norbornyl, decalinyl, 7,7-dimethyl-bicyclo[2.2.1]heptanyl, and the like. Unless otherwise stated specifically in the specification, a cycloalkyl group is optionally substituted.

[0037] "Cycloalkylalkyl" refers to a radical of the formula $–R_b-R_c$ where R_b is an alkylene chain and R_c is one or more cycloalkyl radicals as defined above, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl and the like. A cycloalkylalkyl group may contain a C_1-C_{10} alkylene chain connected to a C_3-C_{12} cycloalkyl radical (*i.e.*, C_3-C_{12} cycloalkylalkyl) or a C_1-C_{10} alkylene chain connected to a C_3-C_6 cycloalkyl radical (*i.e.*, C_3-C_6 cycloalkylalkyl). Unless stated otherwise specifically in the specification, a cycloalkylalkyl group is optionally substituted.

[0038] "Fused" refers to any ring structure described herein which is fused to an existing ring structure in the compounds of the disclosure. When the fused ring is a heterocyclyl ring or a heteroaryl ring, any carbon atom on the existing ring structure which becomes part of the fused heterocyclyl ring or the fused heteroaryl ring is replaced with a nitrogen atom.

[0039] "Halo" or "halogen" refers to bromo, chloro, fluoro, or iodo.

[0040] "Haloalkyl" refers to an alkyl radical, as defined above, that is substituted by one or more halo radicals, as defined above, *e.g.*, trifluoromethyl, difluoromethyl, trichloromethyl, 2,2,2-trifluoroethyl, 1,2-difluoroethyl, 3-bromo-2-fluoropropyl, 1,2-dibromoethyl, and the like. A preferred haloalkyl group includes an alkyl group having one to six carbon atoms and that is substituted by one or more halo radicals (*i.e.*, C_1-C_6 haloalkyl). The halo radicals may be all the same or the halo radicals may be different. Unless stated otherwise specifically in the specification, a haloalkyl group is optionally substituted.

[0041] "Haloalkoxy" refers to a radical of the formula $-OR_a$ where R_a is a haloalkyl radical as defined herein containing one to twelve carbon atoms. A preferred haloalkoxy group includes an alkoxy group having one to six carbon atoms (*i.e.*, C_1-C_6 haloalkoxy) or having one to three carbon atoms (C_1-C_3 haloalkoxy) and that is substituted by one or more halo radicals. The halo radicals may all be the same or the halo radicals may all be different. Unless stated otherwise specifically in the specification, a haloalkoxy group is optionally substituted.

[0042] "Heteroaryl" refers to an aromatic group (e.g., a 5-14 membered ring system) having a single ring, multiple rings, or multiple fused rings, with one or more ring heteroatoms independently selected from nitrogen, oxygen and sulfur. As used herein, heteroaryl includes 1 to 10 ring carbon atoms and 1 to 4 heteroatoms independently selected from nitrogen, oxygen and sulfur within the ring. Preferred heteroaryl groups have a 5- to 10-membered ring system containing one to four heteroatoms selected from nitrogen, oxygen, and sulfur (*i.e.*, a 5- to 10-membered heteroaryl) and a 5- to 6-membered ring system containing one to four heteroatoms selected from nitrogen, oxygen, and sulfur (*i.e.*, a 5- to 6-membered heteroaryl). For purposes of embodiments of this disclosure, the heteroaryl radical may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems. Examples of heteroaryl groups include pyrrolyl, pyrazolyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinazolinyl, quinoxalinyl, quinolinyl, quinuclidinyl, isoquinolinyl, tetrahydroquinolinyl, thiazolyl, thiadiazolyl, triazolyl, tetrazolyl, triazinyl and thiophenyl (i.e., thienyl). A heteroaryl may comprise one or more N-oxide (N-O-) moieties, such as pyridine-N-oxide. Unless stated otherwise specifically in the specification, a heteroaryl group is optionally substituted.

[0043] "Heteroarylalkyl" refers to a radical of the formula $–R_b$-R_c where R_b is an alkylene chain and R_c is one or more heteroaryl radicals as defined above. A heteroarylalkyl group may contain a C_1-C_{10} alkylene chain connected to a 5- to 10-membered heteroaryl group (*i.e.*, 5- to 10-membered heteroarylalkyl) or a C_1-C_{10} alkylene chain connected to a 5- to 6-membered heteroaryl group (*i.e.*, 5- to 6-membered heteroarylalkyl). Unless stated otherwise specifically in the specification, a heteroarylalkyl group is optionally substituted.

[0044] "Heterocyclyl" refers to a saturated or unsaturated cyclic alkyl group, with one or more ring heteroatoms independently selected from nitrogen, oxygen and sulfur. The term "heterocyclyl" includes heterocycloalkenyl groups (*i.e.*, the heterocyclyl group having at least one double bond), bridged-heterocyclyl groups, fused-heterocyclyl groups and spiro-heterocyclyl groups. A heterocyclyl may be a single ring or multiple rings wherein the multiple rings may be fused, bridged or spiro, and may comprise one or more oxo (C=O) or N-oxide (N-O-) moieties. Any non-aromatic ring containing at least one heteroatom is considered a heterocyclyl, regardless of the attachment (*i.e.*, can be bound through a carbon atom or a heteroatom). Further, the term heterocyclyl is intended to encompass any non-aromatic ring containing at least one heteroatom, which ring may be fused to an aryl or heteroaryl ring, regardless of the attachment to the remainder of the molecule. As used herein, heterocyclyl has 1 to 10 ring carbon atoms, 1 to 8 carbon atoms, 1 to 6 carbon atoms, or 1 to 4 carbon atoms, and 1 to 5 ring heteroatoms, 1 to 4 heteroatoms, 1 to 3 heteroatoms, or 1 to 2 heteroatoms independently selected from nitrogen, sulfur and oxygen. Preferred heterocyclyls have five to 10 members in the ring system including one to four heteroatoms selected from nitrogen and oxygen (*i.e.*, 5- to 10-membered heterocyclyl) or five to eight members in the ring system including one to four heteroatoms selected from nitrogen and oxygen (*i.e.*, 5- to 8-membered heterocyclyl). Examples of heterocyclyl groups include dioxolanyl, thienyl[1,3]dithianyl, decahydroisoquinolyl, imidazolinyl, imidazolidinyl, isothiazolidinyl, isoxazolidinyl, morpholinyl, octahydroindolyl, octahydroisoindolyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, oxazolidinyl, piperidinyl, piperazinyl, 4-piperidonyl, pyrrolidinyl, pyrazolidinyl, quinuclidinyl, thiazolidinyl, tetrahydrofuryl, trithianyl, tetrahydropyranyl, thiomorpholinyl, thiamorpholinyl, 1-oxo-thiomorpholinyl and 1,1-dioxo-thiomorpholinyl. Unless stated otherwise specifically in the specification, a hetercyclyl group is optionally substituted.

[0045] "Heterocyclylalkyl" refers to a radical of the formula $-R_b-R_c$ where R_b is an alkylene chain and R_c is one or more heterocyclyl radicals as defined above. A heterocyclylalkyl group may contain a C_1-C_{10} alkylene chain connected to a 5- to 10-membered heterocyclyl radical (*i.e.*, 5- to 10-membered heterocyclylalkyl) or a C_1-C_{10} alkylene chain connected to a 5- to 8-membered heterocyclyl radical (*i.e.*, 5- to 8-membered heterocyclylalkyl). Unless stated otherwise specifically in the specification, a heterocyclylalkyl group is optionally substituted.

[0046] In some embodiments, the term "substituted" as used herein means any of the above groups, or other substituents (*e.g.*, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{12} cycloalkyl, C_3-C_{12} cycloalkylalkyl, aryl, and heteroaryl) wherein at least one hydrogen atom (*e.g.*, 1, 2, 3, or all hydrogen atoms) is replaced by a bond to a non-hydrogen atom such as, but not limited to: a

halogen atom such as F, Cl, Br, and I (*i.e.*, "halo"); an oxygen atom in groups such as hydroxyl groups or alkoxy groups (*e.g.*, alkoxy or haloalkoxy); a nitrogen atom in groups such as amines (*e.g.*, $-NH_2$), amides (*e.g.*, $-(C=O)NH_2$), and nitro; alkyl groups including one or more halogen, such as F, Cl, Br, and I (*e.g.*, haloalkyl); and cyano.

[0047] It is understood that each choice for L, R^{1a}, R^{1b}, R^2, R^3, R^4, R^5, R^6, and R^7 is optionally substituted as described above unless specifically stated otherwise, and provided that all valences are satisfied by the substitution. Specifically, each choice for L, R^{1a}, R^{1b}, R^2, R^3, R^4, R^5, R^6, and R^7 is optionally substituted unless specifically stated otherwise, and provided such substitution results in a stable molecule (*e.g.*, groups such as H and halo are not optionally substituted).

[0048] "Neuroinflammation" or "neuroinflammatory condition" refer to an inappropriate or prolonged inflammation in tissues of the central nervous system (i.e., within the brain and/or spinal cord). Neuroinflammation is mediated by the production of cytokines, chemokines, reactive oxygen species, and/or secondary messengers. In some embodiments, neuroinflammation is distinct from an inflammatory response outside of the central nervous system.

[0049] "Effective amount" or "therapeutically effective amount" of a compound or a composition refers to that amount of the compound or the composition that results in an intended result as desired based on the disclosure herein. Effective amounts can be determined by standard pharmaceutical procedures in cell cultures or experimental animals including, without limitation, by determining the ED_{50} (the dose therapeutically effective in 50% of the population) and the LD_{50} (the dose lethal to 50% of the population). In some embodiments, an effective amount of a compound results in reduction or inhibition of symptoms or a prolongation of survival in a subject (*i.e.*, a human patient). The results may require multiple doses of the compound.

[0050] "Treating" or "treatment" of a disease in a subject refers to 1) preventing the disease from occurring in a patient that is predisposed or does not yet display symptoms of the disease; 2) inhibiting the disease or arresting its development; or 3) ameliorating or causing regression of the disease. As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. For the purposes of this disclosures, beneficial or desired results include, but are not limited to, one or more of the following: decreasing one or more symptoms resulting from the disease or disorder, diminishing the extent of the disease or disorder, stabilizing the disease or disorder (e.g., preventing or delaying the worsening of the disease or disorder), delaying the occurrence or recurrence of the disease or disorder, delaying or slowing the progression of the disease or disorder, ameliorating the disease or disorder state,

providing a remission (whether partial or total) of the disease or disorder, decreasing the dose of one or more other medications required to treat the disease or disorder, enhancing the effect of another medication used to treat the disease or disorder, delaying the progression of the disease or disorder, increasing the quality of life, and/or prolonging survival of a subject. Also encompassed by "treatment" is a reduction of pathological consequence of the disease or disorder. The methods of the invention contemplate any one or more of these aspects of treatment.

[0051] As used herein, the terms "individual(s)", "subject(s)" and "patient(s)" mean any mammal. Examples include, but are not limited to, mice, rats, hamsters, guinea pigs, pigs, rabbits, cats, dogs, goats, sheep, cows, and humans. In some embodiments, the mammal is a human.

[0052] A "therapeutic effect", as that term is used herein, encompasses a therapeutic benefit and/or a prophylactic benefit as described herein. A therapeutic effect includes delaying or eliminating the appearance of a disease or condition; delaying or eliminating the onset of symptoms of a disease or condition; slowing, halting, or reversing the progression of a disease or condition; causing partial or complete regression of a disease or condition; or any combination thereof.

[0053] The terms "co-administration", "administered in combination with", and their grammatical equivalents, as used herein, encompass administration of two or more agents to an animal, including humans, so that both agents and/or their metabolites are present in the subject at the same time. Co-administration includes simultaneous administration in separate compositions, administration at different times in separate compositions, or administration in a composition in which both agents are present.

[0054] "Pharmaceutically acceptable" refers to compounds, salts, compositions, dosage forms and other materials which are useful in preparing a pharmaceutical composition that is suitable for veterinary or human pharmaceutical use.

[0055] "Pharmaceutically acceptable salt" includes both acid and base addition salts.

[0056] "Pharmaceutically acceptable acid addition salt" refers to those salts which retain the biological effectiveness and properties of the free bases, which are not biologically or otherwise undesirable, and which are formed with inorganic acids such as, but are not limited to, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as, but not limited to, acetic acid, 2,2-dichloroacetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, 4-acetamidobenzoic acid, camphoric acid, camphor-10-sulfonic acid, capric acid, caproic acid, caprylic acid, carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid,

ethanesulfonic acid, 2-hydroxyethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, 2-oxo-glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, isobutyric acid, lactic acid, lactobionic acid, lauric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, mucic acid, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, 1-hydroxy-2-naphthoic acid, nicotinic acid, oleic acid, orotic acid, oxalic acid, palmitic acid, pamoic acid, propionic acid, pyroglutamic acid, pyruvic acid, salicylic acid, 4-aminosalicylic acid, sebacic acid, stearic acid, succinic acid, tartaric acid, thiocyanic acid, *p*-toluenesulfonic acid, trifluoroacetic acid, undecylenic acid, and the like.

[0057] "Pharmaceutically acceptable base addition salt" refers to those salts which retain the biological effectiveness and properties of the free acids, which are not biologically or otherwise undesirable. These salts are prepared from addition of an inorganic base or an organic base to the free acid. Salts derived from inorganic bases include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Preferred inorganic salts are the ammonium, sodium, potassium, calcium, and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as ammonia, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, diethanolamine, ethanolamine, deanol, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, benethamine, benzathine, ethylenediamine, glucosamine, methylglucamine, theobromine, triethanolamine, tromethamine, purines, piperazine, piperidine, *N*-ethylpiperidine, polyamine resins and the like. Particularly preferred organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline, and caffeine.

[0058] In some embodiments, pharmaceutically acceptable salts include quaternary ammonium salts such as quaternary amine alkyl halide salts (*e.g.*, methyl bromide).

[0059] As used herein, "therapeutic agent" refers to a biological, pharmaceutical, or chemical compound or other moiety. Non-limiting examples include a simple or complex organic or inorganic molecule, a peptide, a protein, an oligonucleotide, an antibody, an antibody derivative, antibody fragment, a vitamin derivative, a carbohydrate, a toxin, or a chemotherapeutic compound. Various compounds can be synthesized, for example, small molecules and oligomers (*e.g.*, oligopeptides and oligonucleotides), and synthetic organic compounds based on various core structures. In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like.

[0060] The term "*in vivo*" refers to an event that takes place in a subject's body.

[0061] Embodiments of the disclosure are also meant to encompass all pharmaceutically acceptable compounds of Formula (I) being isotopically-labelled by having one or more atoms replaced by an atom having a different atomic mass or mass number (*i.e.*, an "isotopic form" of a compound of Formula (I)). Examples of isotopes that can be incorporated into the compounds of Formula (I) include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine, chlorine, and iodine, such as ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{13}N, ^{15}N, ^{15}O, ^{17}O, ^{18}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, ^{36}Cl, ^{123}I, and ^{125}I, respectively. These radiolabeled compounds could be useful to help determine or measure the effectiveness of the compounds, by characterizing, for example, the site or mode of action, or binding affinity to pharmacologically important site of action. Certain isotopically-labeled compounds of Formula (I), for example, those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The radioactive isotopes tritium, *i.e.*, ^{3}H, and carbon-14, *i.e.*, ^{14}C, are particularly useful for this purpose in view of their ease of incorporation and ready means of detection.

[0062] Substitution with heavier isotopes such as deuterium, *i.e.*, ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements, and hence are preferred in some circumstances.

[0063] Substitution with positron emitting isotopes, such as ^{11}C, ^{18}F, ^{15}O and ^{13}N, can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy. Isotopically-labeled compounds of Formula (I) can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the Examples as set out below using an appropriate isotopically-labeled reagent in place of the non-labeled reagent previously employed.

[0064] Certain embodiments are also meant to encompass the *in vivo* metabolic products of the disclosed compounds. Such products may result from, for example, the oxidation, reduction, hydrolysis, amidation, esterification, and the like of the administered compound, primarily due to enzymatic processes. Accordingly, the embodiments include compounds produced by a process comprising administering a compound of this disclosure to a mammal for a period of time sufficient to yield a metabolic product thereof. Such products are typically identified by administering a radiolabeled compound of the disclosure in a detectable dose to an animal, such as rat, mouse, guinea pig, monkey, or to human, allowing sufficient time for metabolism to occur, and isolating its conversion products from the urine, blood or other biological samples.

[0065] "Stable compound" and "stable structure" are meant to indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

[0066] Often crystallizations produce a solvate of the compound of the disclosure. As used herein, the term "solvate" refers to an aggregate that comprises one or more molecules of a compound of Formula (I) with one or more molecules of solvent. In some embodiments, the solvent is water, in which case the solvate is a hydrate. Alternatively, in other embodiments, the solvent is an organic solvent. Thus, the compounds of Formula (I) may exist as a hydrate, including a monohydrate, dihydrate, hemihydrate, sesquihydrate, trihydrate, tetrahydrate and the like, as well as the corresponding solvated forms. In some aspects, the compound of Formula (I) is a true solvate, while in other cases, the compound of the disclosure merely retains adventitious water or is a mixture of water plus some adventitious solvent.

[0067] "Optional" or "optionally" means that the subsequently described event of circumstances may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted aryl" means that the aryl radical may or may not be substituted and that the description includes both substituted aryl radicals and aryl radicals having no substitution. Polymers or similar indefinite structures arrived at by defining substituents with further substituents appended ad infinitum (e.g., a substituted aryl having a substituted alkyl which is itself substituted with a substituted aryl group, which is further substituted by a substituted heteroalkyl group, etc.) are not intended for inclusion herein. Similarly, the above definitions are not intended to include impermissible substitution patterns (e.g., methyl substituted with 5 fluorines or heteroaryl groups having two adjacent oxygen ring atoms). Such impermissible substitution patterns are well known to the skilled artisan.

[0068] A "pharmaceutical composition" or "pharmaceutically acceptable composition" refers to a formulation of a compound of the disclosure and a medium generally accepted in the art for the delivery of the biologically active compound to mammals, *e.g.*, humans. Such a medium includes all pharmaceutically acceptable carriers, diluents, or excipients therefor.

[0069] "Pharmaceutically acceptable carrier, diluent or excipient" includes, without limitation, any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans or domestic animals.

[0070] The compounds of Formula (I), or a pharmaceutically acceptable salt or isotopic form thereof, may contain one or more centers giving rise to geometric asymmetry and may thus provide enantiomers, diastereomers, and other stereoisomeric forms that are defined, in terms of absolute stereochemistry, as (*R*)- or (*S*)- or, as (D)- or (L)- for amino acids. Embodiments thus include all such possible isomers, as well as their racemic and optically pure forms. Optically

active (+) and (-), (*R*)- and (*S*)-, or (D)- and (L)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques, for example, chromatography and fractional crystallization. Conventional techniques for the preparation/isolation of individual enantiomers include chiral synthesis from a suitable optically pure precursor or resolution of the racemate (or the racemate of a salt or derivative) using, for example, chiral high pressure liquid chromatography (HPLC). When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers. Likewise, all tautomeric forms are also intended to be included.

[0071] A "stereoisomer" refers to a compound made up of the same atoms bonded by the same bonds but having different three-dimensional structures, which are not interchangeable. The present disclosure contemplates various stereoisomers and mixtures thereof and includes "enantiomers", which refers to two stereoisomers whose molecules are non-superimposable mirror images of one another.

[0072] "Diastereoisomers" are stereoisomers that have at least two asymmetric atoms, but which are not mirror-images of each other.

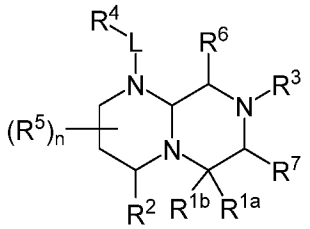
[0073] A "tautomer" refers to a proton shift from one atom of a molecule to another atom of the same molecule. Embodiments thus include tautomers of the disclosed compounds.

[0074] The chemical naming protocol and structure diagrams used herein are a modified form of the I.U.P.A.C. nomenclature system, using the ACD/Name Version 9.07 software program and/or ChemDraw Ultra Version 11.0.1 software naming program (CambridgeSoft). For complex chemical names employed herein, a substituent group is typically named before the group to which it attaches. For example, cyclopropylethyl comprises an ethyl backbone with a cyclopropyl substituent. Except as described below, all bonds are identified in the chemical structure diagrams herein, except for all bonds on some carbon atoms, which are assumed to be bonded to sufficient hydrogen atoms to complete the valency.

[0075] Although various features of the invention may be described in the context of a single embodiment, the features may also be provided separately or in any suitable combination. Conversely, although the invention may be described herein in the context of separate embodiments for clarity, the invention may also be implemented in a single embodiment.

Compounds

[0076] In one aspect, provided herein is a compound of Formula (I):



(I)

or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, wherein:

L is a direct bond, -C(=O)-, -(CR^aR^b)$_m$-C(=O)-, -C(=O)-(CR^aR^b)$_m$-, or -(CR^aR^b)$_m$-;

each R^a and R^b is independently H, C_1-C_6 alkyl, C_2-C_6 alkenyl, or C_2-C_6 alkynyl;

R^{1a} and R^{1b} are independently H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, halo, or C_6-C_{10} arylalkyl;

R^2 is H, oxo, or thioxo;

R^3 is C_2-C_6 alkyl, C_3-C_6 alkenyl, C_3-C_6 alkynyl, C_3-C_{12} cycloalkyl, C_3-C_6 cycloalkylalkyl, C_6-C_{10} arylalkyl, 5- to 10-membered heteroarylalkyl, or 5- to 10-membered heterocyclylalkyl, wherein the 5- to 10-membered heteroarylalkyl or 5- to 10-membered heterocyclylalkyl contains 1-3 heteroatoms selected from nitrogen and oxygen;

R^4 is C_6-C_{10} aryl, 5- to 10-membered heteroaryl, or 5- to 10-membered heterocyclyl, wherein the 5- to 10-membered heteroaryl or 5- to 10-membered heterocyclyl contains 1-3 heteroatoms selected from nitrogen and oxygen;

each R^5 is independently C_1-C_6 alkyl, oxo, or halo;

R^6 is H, C_1-C_6 alkyl, or oxo;

R^7 is H or oxo;

m is 1 or 2; and

n is an integer from 0 to 3;

wherein each C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{12} cycloalkyl, C_3-C_{12} cycloalkylalkyl, C_6-C_{10} aryl, C_6-C_{10} arylalkyl, 5- to 10-membered heteroaryl, 5- to 10-membered heteroarylalkyl, 5- to 10-membered heterocyclyl, and 5- to 10-membered heterocyclylalkyl is optionally substituted with one to five substituents selected from hydroxyl, halo, amino, C_1-C_6 haloalkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkoxy, cyano, -(C=O)NH_2, nitro, -SO_2(C_1-C_6 alkyl), and -CO_2H.

[0077] In some embodiments, L is a direct bond. In some embodiments, L is -C(=O)- or -(CR^aR^b)$_m$-. In some embodiments, L is -C(=O)-. In some embodiments, L is -(CR^aR^b)$_m$-. In some embodiments, L is -(CR^aR^b)$_m$-C(=O)- or -C(=O)-(CR^aR^b)$_m$-. In some embodiments, L is -(CR^aR^b)$_m$-C(=O)-. In some embodiments, L is -C(=O)-(CR^aR^b)$_m$-.

[0078] In some embodiments, each R^a and R^b is independently H, C_1-C_6 alkyl, C_2-C_6 alkenyl, or C_2-C_6 alkynyl. In some embodiments, each R^a and R^b is independently H, C_1-C_3 alkyl, C_2-C_4 alkenyl, or C_2-C_4 alkynyl. In some embodiments, R^a and R^b are each H. In some embodiments, R^a is H. In some embodiments, R^a is C_1-C_6 alkyl, such as methyl, ethyl, or propyl. In some embodiments, R^a is C_2-C_6 alkenyl, such as vinyl or propenyl. In some embodiments, R^a is C_2-C_6 alkynyl, such as ethynyl or propynyl. In some embodiments, R^b is H. In some embodiments, R^b is C_1-C_6 alkyl, such as methyl, ethyl, or propyl. In some embodiments, R^b is C_2-C_6 alkenyl, such as vinyl or propenyl. In some embodiments, R^b is C_2-C_6 alkynyl, such as ethynyl or propynyl.

[0079] In some embodiments, R^{1a} and R^{1b} are independently H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, halo, or C_6-C_{10} arylalkyl. In some embodiments, R^{1a} is H. In some embodiments, R^{1a} is C_1-C_6 alkyl, such as methyl, ethyl, or propyl. In some embodiments, R^{1a} is C_2-C_6 alkenyl, such as vinyl or propenyl. In some embodiments, R^{1a} is C_2-C_6 alkynyl, such as ethynyl or propynyl. In some embodiments, R^{1a} is C_1-C_6 alkoxy, such as methoxy, ethoxy, or propoxy. In some embodiments, R^{1a} is halo, such as fluoro, chloro, or bromo. In some embodiments, R^{1a} is C_6-C_{10} arylalkyl, such as benzyl. In some embodiments, R^{1b} is H. In some embodiments, R^{1b} is C_1-C_6 alkyl, such as methyl, ethyl, or propyl. In some embodiments, R^{1b} is C_2-C_6 alkenyl, such as vinyl or propenyl. In some embodiments, R^{1b} is C_2-C_6 alkynyl, such as ethynyl or propynyl. In some embodiments, R^{1b} is C_1-C_6 alkoxy, such as methoxy, ethoxy, or propoxy. In some embodiments, R^{1b} is halo, such as fluoro, chloro, or bromo. In some embodiments, R^{1b} is C_6-C_{10} arylalkyl, such as benzyl.

[0080] In some embodiments, R^{1a} and R^{1b} are each independently H; C_1-C_6 alkyl optionally substituted with 1-3 substituents selected from halo, $-CO_2H$, and $-C(=O)NH_2$; C_1-C_6 alkoxy; halo; or C_6-C_{10} arylalkyl optionally substituted by 1-3 substituents selected from halo and amino. In some embodiments, R^{1a} is C_1-C_6 alkyl substituted with 1-3 halo, such as fluoro or chloro. In some embodiments, R^{1a} is C_1-C_6 alkyl substituted with 1-3 $-CO_2H$ groups. In some variations, R^{1a} is C_1-C_3 alkyl substituted with 1-2 CO_2H groups, such as $-CH_2CO_2H$ or $-CH_2CH_2CO_2H$. In some embodiments, R^{1a} is C_1-C_6 alkyl substituted with 1-3 $-C(=O)NH_2$ groups. In some embodiments, R^{1a} is C_1-C_3 alkyl substituted with 1-2 $-C(=O)NH_2$ groups, such as $-CH_2C(=O)NH_2$ or $-CH_2CH_2C(=O)NH_2$. In some embodiments, R^{1a} is C_6-C_{10} arylalkyl substituted by 1-3 substituents selected from halo and amino. In some embodiments, R^{1a} is C_6-C_{10} arylalkyl substituted by 1-3 halo, such as fluoro, chloro, or bromo. In some embodiments, R^{1a} is C_6-C_{10} arylalkyl substituted by 1-3 amino. In some embodiments, R^{1b} is C_1-C_6 alkyl substituted with 1-3 halo, such as fluoro or chloro. In some embodiments, R^{1b} is C_1-C_6 alkyl substituted with 1-3 $-CO_2H$ groups. In some variations, R^{1b} is C_1-C_3 alkyl substituted with 1-2

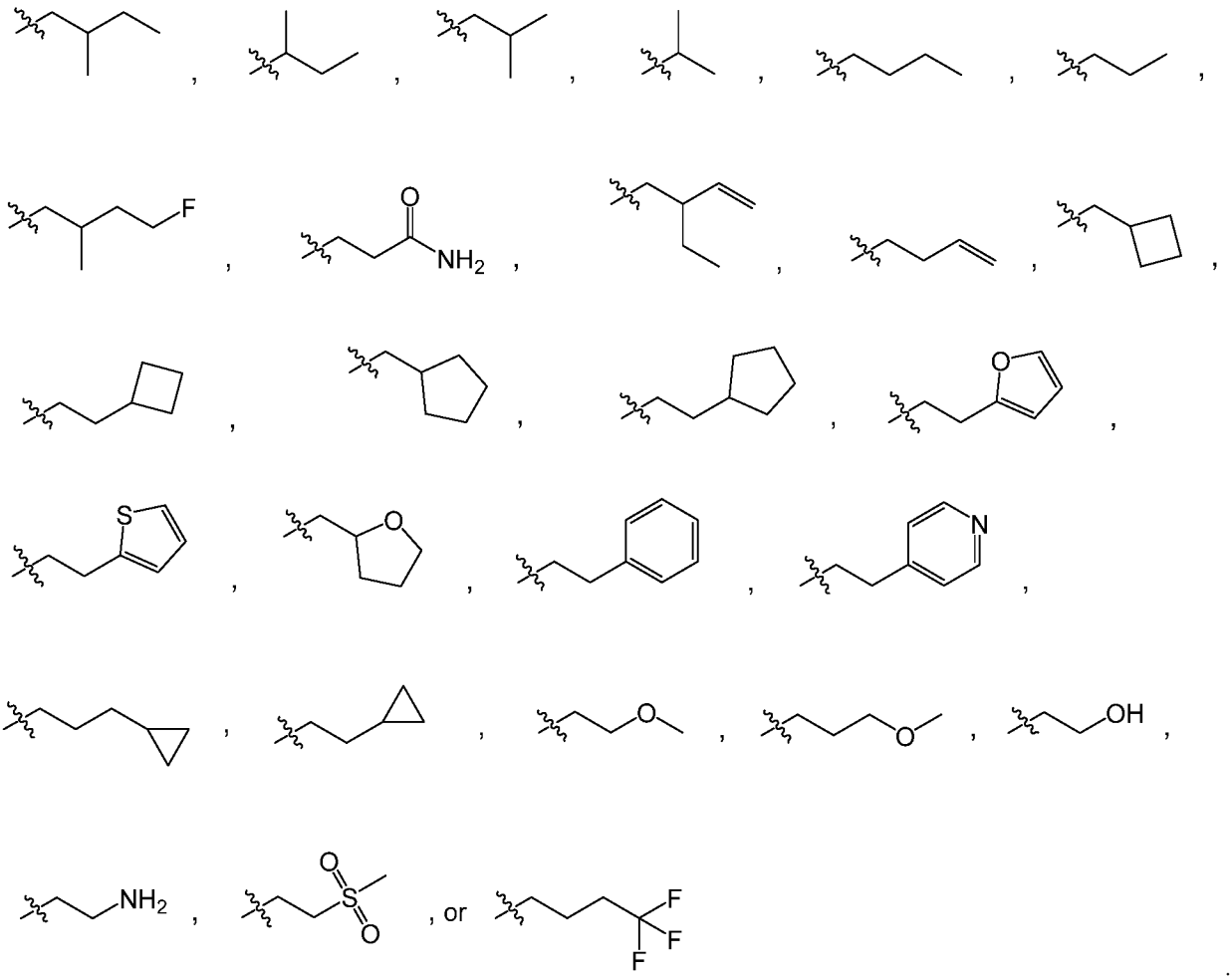
CO_2H groups, such as $-CH_2CO_2H$ or $-CH_2CH_2CO_2H$. In some embodiments, R^{1b} is C_1-C_6 alkyl substituted with 1-3 $-C(=O)NH_2$ groups. In some embodiments, R^{1b} is C_1-C_3 alkyl substituted with 1-2 $-C(=O)NH_2$ groups, such as $-CH_2C(=O)NH_2$ or $-CH_2CH_2C(=O)NH_2$. In some embodiments, R^{1b} is C_6-C_{10} arylalkyl substituted by 1-3 substituents selected from halo and amino. In some embodiments, R^{1b} is C_6-C_{10} arylalkyl substituted by 1-3 halo, such as fluoro, chloro, or bromo. In some embodiments, R^{1b} is C_6-C_{10} arylalkyl substituted by 1-3 amino. In some embodiments, R^{1a} and R^{1b} are each independently H, methyl, fluoro, 2-methylbutyl, $-CH_2F$, methoxy, $-CH_2CO_2H$, $-CH_2C(=O)NH_2$, benzyl, or 4-aminobenzyl. In some embodiments, R^{1a} and R^{1b} are each independently H or C_1-C_3 alkyl. In some embodiments, R^{1a} is methyl and R^{1b} is H. In some embodiments, R^{1a} and R^{1b} are each H. In some embodiments, one of R^{1a} and R^{1b} is H and the other is C_1-C_3 alkyl, such as methyl.

[0081] In some embodiments, R^2 is H, oxo, or thioxo. In some embodiments, R^2 is H. In some embodiments, R^2 is oxo. In some embodiments, R^2 is thioxo.

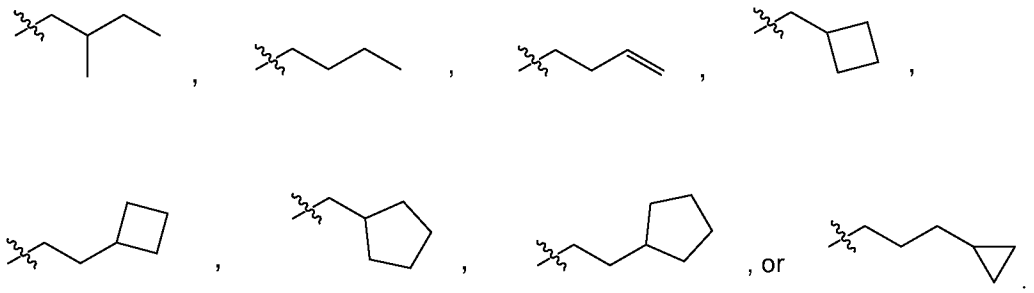
[0082] In some embodiments, R^3 is C_3-C_6 alkyl, C_3-C_6 alkenyl, C_3-C_6 alkynyl, C_3-C_{12} cycloalkyl, C_3-C_6 cycloalkylalkyl, C_6-C_{10} arylalkyl, 5- to 10-membered heteroarylalkyl, or 5- to 10-membered heterocyclylalkyl, wherein the 5- to 10-membered heteroarylalkyl or 5- to 10-membered heterocyclylalkyl contains 1-3 heteroatoms selected from nitrogen and oxygen. In some embodiments, R^3 is C_3-C_6 alkyl, such as propyl, butyl, pentyl, or hexyl. In some embodiments, R^3 is C_4-C_6 alkyl. In some embodiments, R^3 is C_3-C_6 alkenyl. In some embodiments, R^3 is C_4-C_6 alkenyl. In some embodiments, R^3 is C_3-C_6 alkynyl. In some embodiments, R^3 is C_4-C_6 alkynyl. In some embodiments, R^3 is C_3-C_{12} cycloalkyl, such as cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl. In some embodiments, R^3 is C_3-C_6 cycloalkyl. In some embodiments, R^3 is C_3-C_6 cycloalkylalkyl, such as $-(CH_2)_{1\text{-}3}$(C_3-C_6 cycloalkyl). In some embodiments, R^3 is C_6-C_{10} arylalkyl, such as benzyl. In some embodiments, R^3 is 5- to 10-membered heteroarylalkyl, such as $-(CH_2)_{1\text{-}3}$(5- to 10-membered heteroaryl) or $-(CH_2)_{1\text{-}3}$(5- to 6-membered heteroaryl). In some embodiments, the 5- to 10-membered heteroarylalkyl contains 1-2 nitrogen atoms. In some embodiments, R^3 is 5- to 10-membered heterocyclylalkyl, such as $-(CH_2)_{1\text{-}3}$(5- to 10-membered heterocyclyl) or $-(CH_2)_{1\text{-}2}$(5- to 6-membered heterocyclyl). In some embodiments, the 5- to 10-membered heterocyclylalkyl contains 1-2 nitrogen atoms.

[0083] In some embodiments, R^3 is C_3-C_6 alkyl optionally substituted by 1-3 substituents selected from halo and $-C(=O)NH_2$, C_2-C_6 alkenyl, or C_3-C_6 cycloalkylalkyl. In some embodiments, R^3 is C_2-C_6 alkyl optionally substituted by 1-3 substituents selected from halo, C_1-C_3 alkoxy, hydroxy, $-NH_2$, $-SO_2(C_1\text{-}C_3$ alkyl), and $-C(=O)NH_2$; C_2-C_6 alkenyl; C_3-C_6 cycloalkylalkyl; 5- to 6-membered heteroarylalkyl; 5- to 6-membered heterocyclylalkyl; or C_6

arylalkyl. In some embodiments, R^3 is C_2 alkyl substituted by 1-3 substituents selected from C_1-C_3 alkoxy, hydroxy, $-NH_2$, and $-SO_2(C_1$-C_3 alkyl). In some embodiments, R^3 is:



In some embodiments, R^3 is:

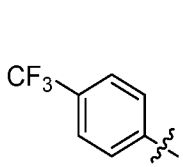
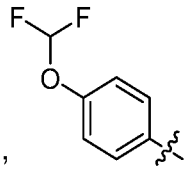
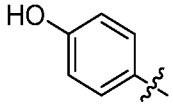
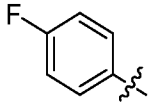
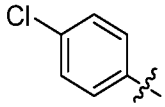
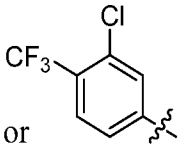


In some embodiments, R^3 is 2-methylbutyl.

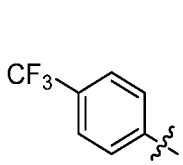
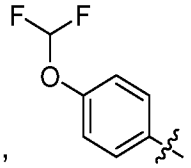
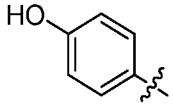
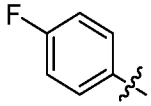
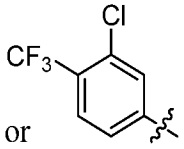
[0084] In some embodiments, R^4 is C_6-C_{10} aryl, 5- to 10-membered heteroaryl, or 5- to 10-membered heterocyclyl, wherein the 5- to 10-membered heteroaryl or 5- to 10-membered heterocyclyl contains 1-3 heteroatoms selected from nitrogen and oxygen. In some embodiments, R^4 is C_6-C_{10} aryl, such as phenyl. In some embodiments, R^4 is 5- to 10-membered heteroaryl containing 1-2 nitrogen atoms. In some embodiments, R^4 is 5- to 10-membered heterocyclyl. In some embodiments, R^4 is 5- to 9-membered heterocyclyl containing 1-2 nitrogen atoms. In some embodiments, R^4 is 5- to 9-membered heterocyclyl containing 1-2

oxygen atoms. In some embodiments, R^4 is 5- to 9-membered heterocyclyl containing 1 nitrogen atom and 1 oxygen atom.

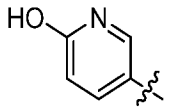
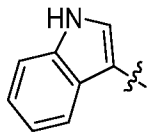
[0085] In some embodiments, R^4 is C_6-C_{10} aryl optionally substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy. In some embodiments, R^4 is phenyl substituted with 1-3 substituents selected from -CF_3, -$OCHF_2$, -OH, fluoro, and chloro. In some embodiments, R^4 is:

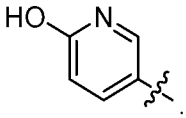
In some embodiments, R^4 is:

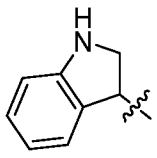
[0086] In some embodiments, R^4 is 5- to 10-membered heteroaryl optionally substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy. In some embodiments, R^4 is pyridyl or indolyl optionally substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy. In some embodiments, R^4 is

. In some embodiments, R^4 is pyridyl substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy. In some embodiments, R^4 is

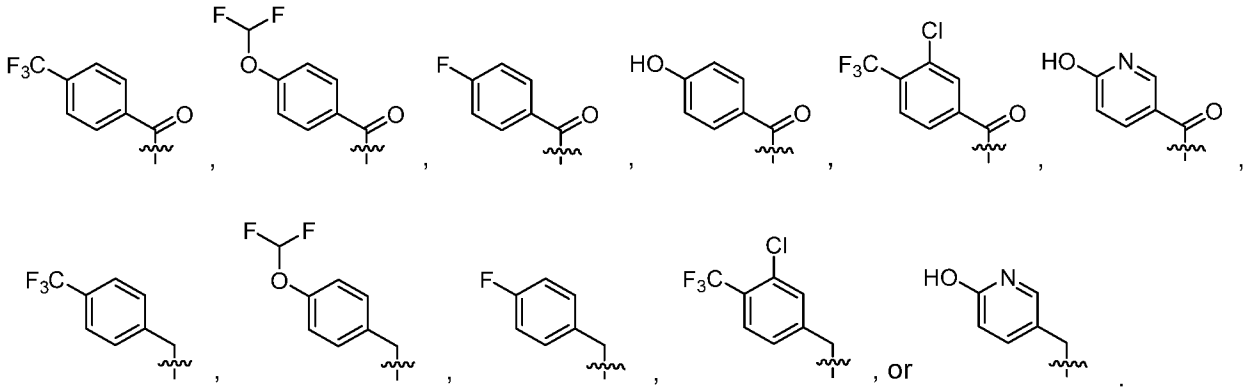


. In some embodiments, R^4 is 5- to 10-membered heterocyclyl optionally substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy. In some embodiments, R^4 is indolinyl.



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[0087] In some embodiments, -L-R^4 is -CH_2(phenyl) or -C(O)(phenyl), wherein the phenyl is substituted by 1-3 substituents selected from C_1-C_3 haloalkyl, C_1-C_3 haloalkoxy, halo, and hydroxy. In some embodiments, -L-R^4 is -CH_2(pyridyl) or -C(O)(pyridyl), wherein the pyridyl is substituted by 1-3 substituents selected from C_1-C_3 haloalkyl, C_1-C_3 haloalkoxy, halo, and hydroxy. In some embodiments, -L-R^4 is:



[0088] In some embodiments, each R^5 is independently C_1-C_6 alkyl, oxo, or halo. In some embodiments, R^5 is C_1-C_6 alkyl, such as methyl, ethyl, or propyl. In some embodiments, R^5 is oxo. In some embodiments, R^5 is halo, such as fluoro, chloro, or bromo. In some embodiments, R^5 is oxo or halo. In some embodiments, R^5 is oxo or fluoro.

[0089] In some embodiments, R^6 is H, C_1-C_6 alkyl, or oxo. In some embodiments, R^6 is H. In some embodiments, R^6 is C_1-C_6 alkyl, such as methyl, ethyl, or propyl. In some embodiments, R^6 is oxo.

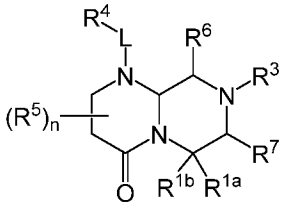
[0090] In some embodiments, R^7 is H or oxo. In some embodiments, R^7 is H. In some embodiments, R^7 is oxo.

[0091] In some embodiments, m is 1. In other embodiments, m is 2.

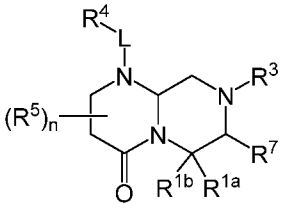
[0092] In some embodiments, n is 0. In other embodiments, n is an integer from 1 to 3. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3.

[0093] In any embodiments of Formula (I), or variations thereof, each C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{12} cycloalkyl, C_3-C_{12} cycloalkylalkyl, C_6-C_{10} aryl, C_6-C_{10} arylalkyl, 5- to 10-membered heteroaryl, 5- to 10-membered heteroarylalkyl, 5- to 10-membered heterocyclyl, and 5- to 10-membered heterocyclylalkyl is optionally substituted with one to three substituents selected from hydroxyl, halo (such as fluoro, chloro, or bromo), amino, C_1-C_6 haloalkyl (such as $-CF_3$ or $-CHF_2$), C_1-C_6 alkoxy (such as methoxy or ethoxy), C_1-C_6 haloalkoxy (such as $-OCHF_2$ or $-OCF_3$), and $-(C=O)NH_2$.

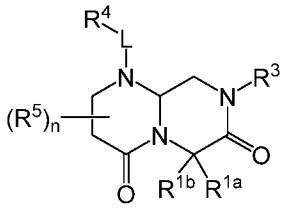
[0094] In some embodiments, the compound of Formula (I) is a compound of Formula (II), (IIa), (IIb), (IIc), (IId), or (IIe):



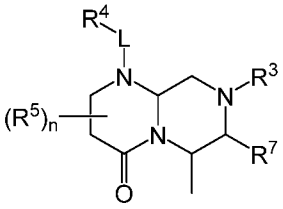
(II)



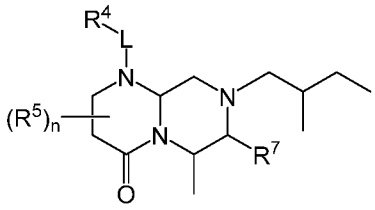
(IIa)



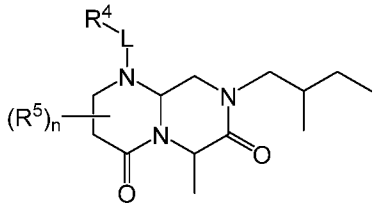
(IIb)



(IIc)



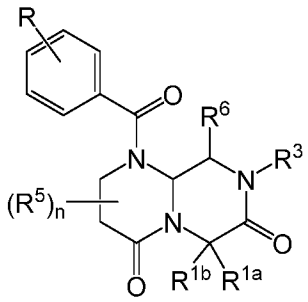
(IId)



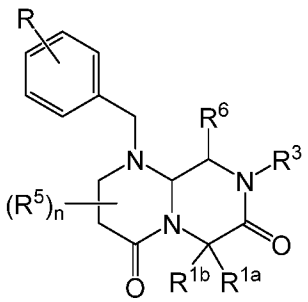
(IIe)

or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, wherein L, R^{1a}, R^{1b}, R^3, R^4, R^5, R^6, R^7, and n are as described for Formula (I). In some embodiments, the compound is of Formula (II) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, the compound is of Formula (IIa) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, the compound is of Formula (IIb) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, the compound is of Formula (IIc) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, the compound is of Formula (IId) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, the compound is of Formula (IIe) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof.

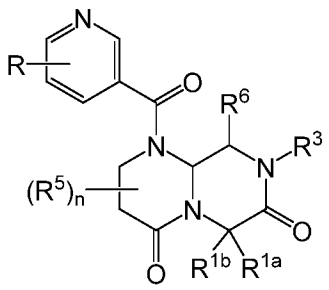
[0095] In some embodiments, the compound of Formula (I) is a compound of Formula (IIIa), (IIIb), (IIIc), or (IIId):



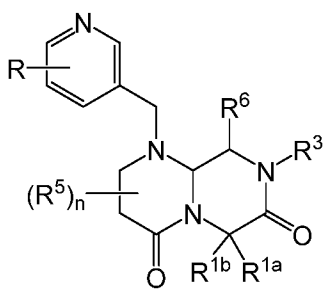
(IIIa) ,



(IIIb) ,



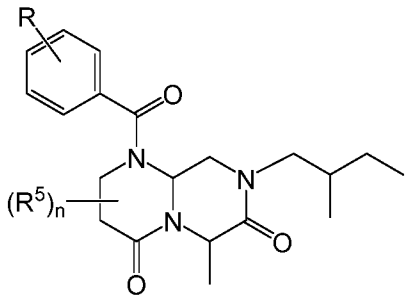
(IIIc) , or



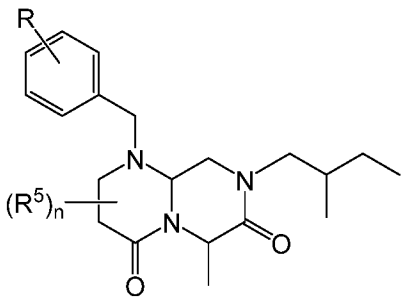
(IIId) ,

or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, wherein R^{1a}, R^{1b}, R^3, R^5, R^6, and n are as described for Formula (I), and R represents one or more optional substituents, such as hydroxyl, halo, amino, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, as described for Formula (I). In some embodiments, the compound is of Formula (IIIa) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, the compound is of Formula (IIIb) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, the compound is of Formula (IIIc) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, the compound is of Formula (IIId) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof.

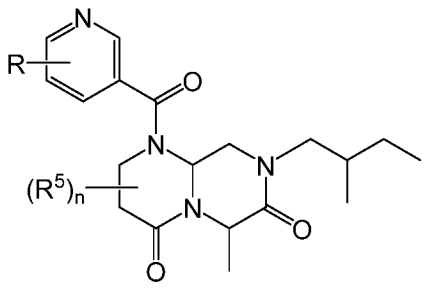
[0096] In some embodiments, the compound of Formula (I) is a compound of Formula (IVa), (IVb), (IVc), or (IVd):



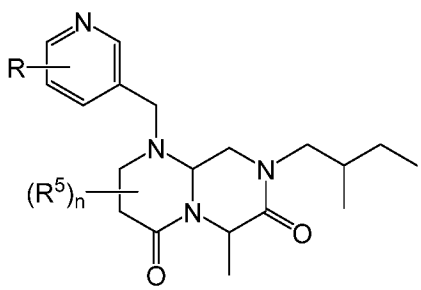
(IVa) ,



(IVb) ,



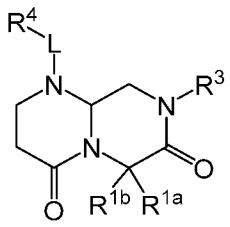
(IVc) , or



(IVd)

or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, wherein R^5 and n are as described for Formula (I), and R represents one or more optional substituents, such as hydroxyl, halo, amino, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, as described for Formula (I). In some embodiments, the compound is of Formula (IVa) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, the compound is of Formula (IVb) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, the compound is of Formula (IVc) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, the compound is of Formula (IVd) or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof.

[0097] In some embodiments, the compound of Formula (I) is a compound of Formula (V):



(V)

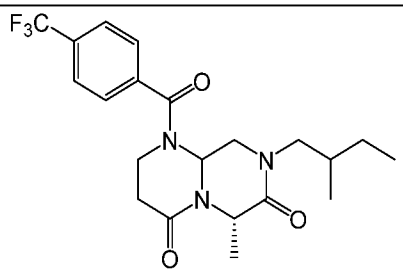
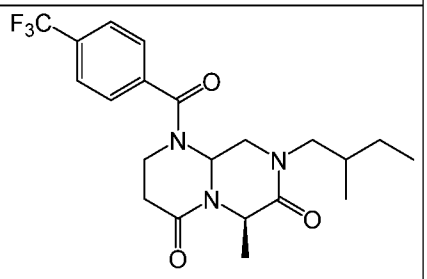
or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, wherein L, R^{1a}, R^{1b}, R^3, and R^4 are as described for Formula (I). In some embodiments, L is -C(=O)- or -CH_2-; R^{1a} and R^{1b} are independently H or C_1-C_3 alkyl optionally substituted with -CO_2H; R^3 is C_4-C_5 alkyl, C_4-C_5 alkenyl, or C_1-C_3 alkyl substituted with C_3-C_5 cycloalkyl; and R^4 is phenyl or

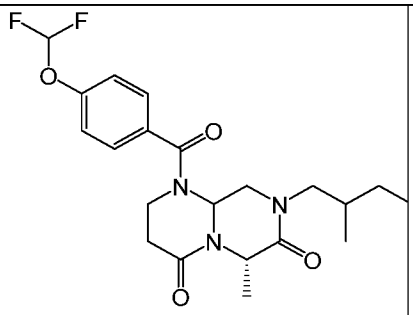
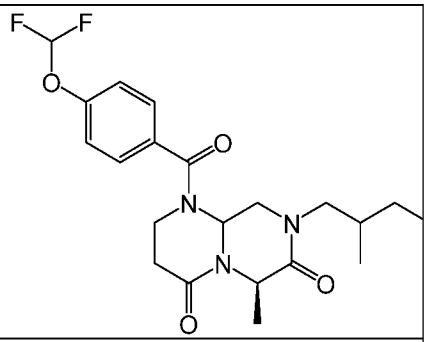
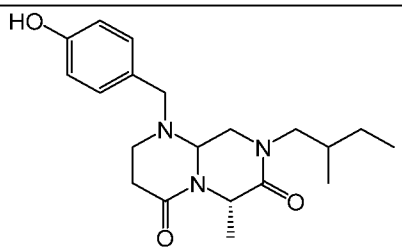
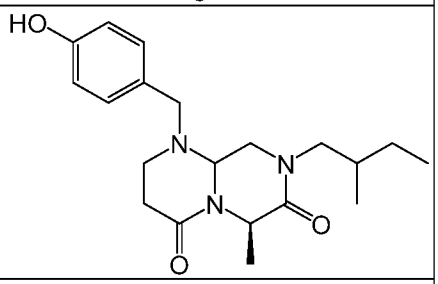
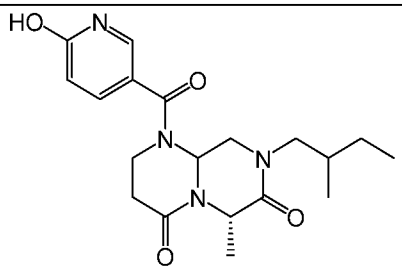
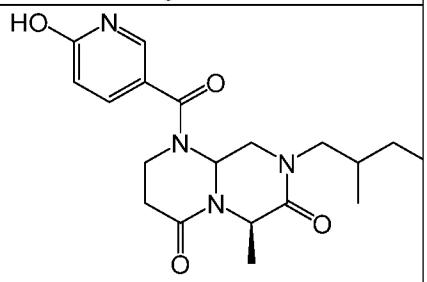
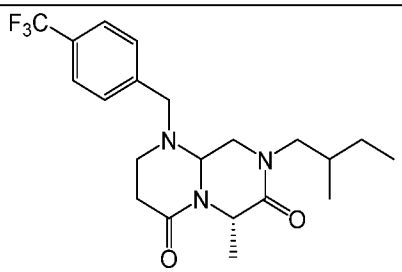
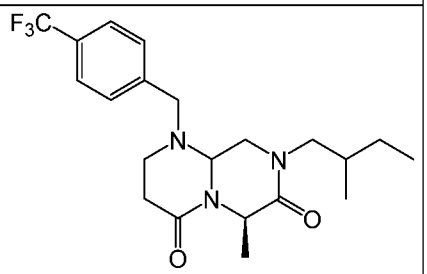
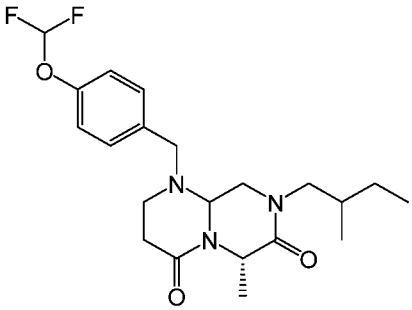
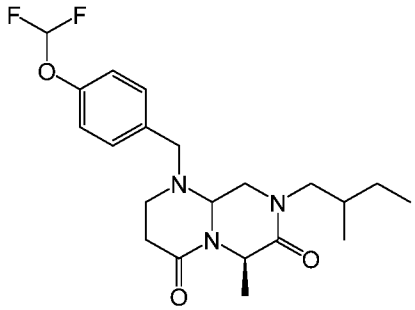
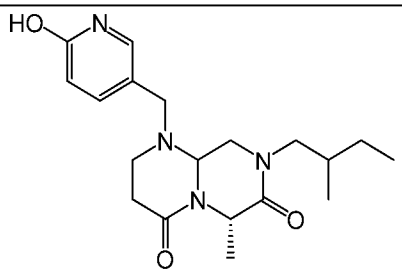
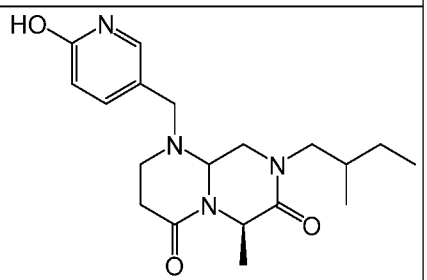
pyridyl substituted with 1-3 substituents selected from -CF_3, -$OCHF_2$, -OH, fluoro, and chloro. In some variations, one of R^{1a} and R^{1b} is H and the other is C_1-C_3 alkyl, such as methyl.

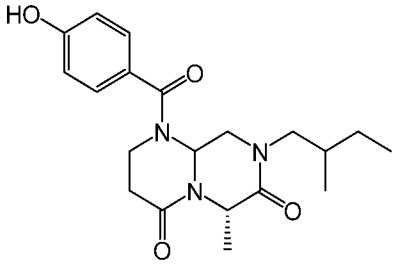
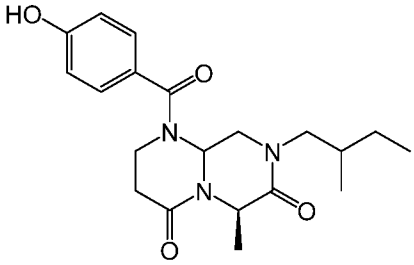
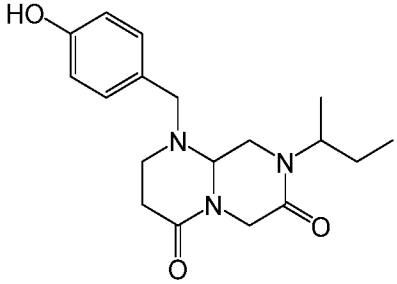
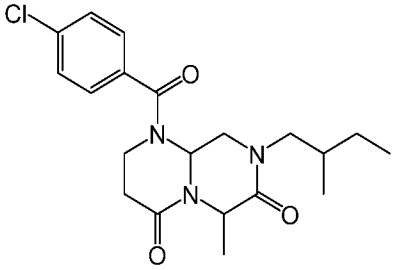
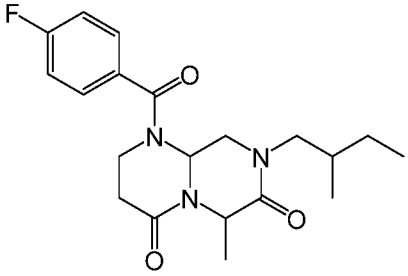
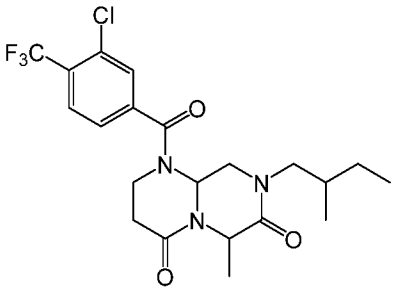
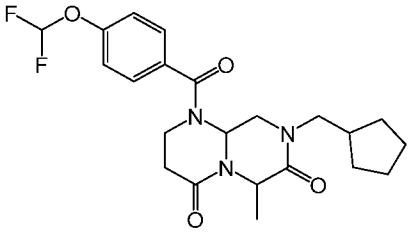
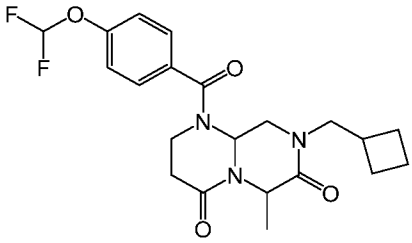
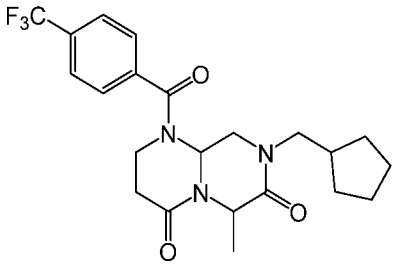
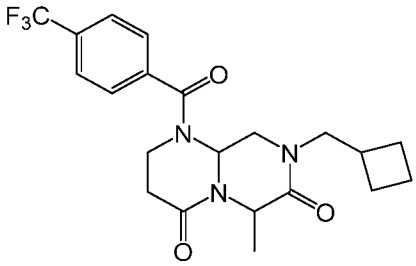
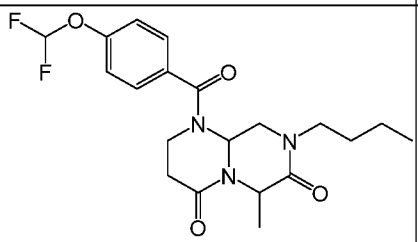
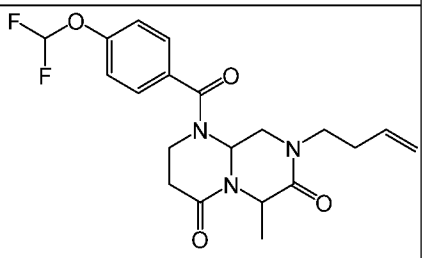
[0098] In the descriptions herein, it is understood that every description, variation, embodiment, or aspect of a moiety may be combined with every description, variation, embodiment, or aspect of other moieties the same as if each and every combination of descriptions is specifically and individually listed. For example, every description, variation, embodiment, or aspect provided herein with respect to L of Formula (I) may be combined with every description, variation, embodiment, or aspect of R^{1a}, R^{1b}, R^2, R^3, R^4, R^5, R^6, R^7, and n the same as if each and every combination were specifically and individually listed. It is also understood that all descriptions, variations, embodiments, or aspects of Formula (I), where applicable, apply equally to other formulae detailed herein, and are equally described, the same as if each and every description, variation, embodiment, or aspect were separately and individually listed for all formulae. For example, all descriptions, variations, embodiments, or aspects of Formula (I), where applicable, apply equally to any of the formulae as detailed herein, such as Formulae (II), (IIa), (IIb), (IIc), (IId), (IIe), (IIIa), (IIIb), (IIIc), (IIId), (IVa), (IVb), (IVc), (IVd), and (V), and are equally described, the same as if each and every description, variation, embodiment, or aspect were separately and individually listed for all formulae.

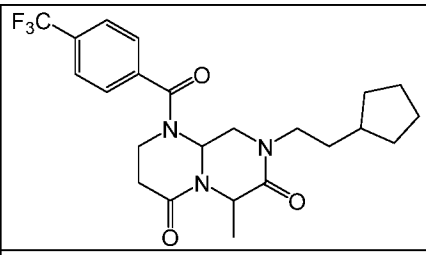
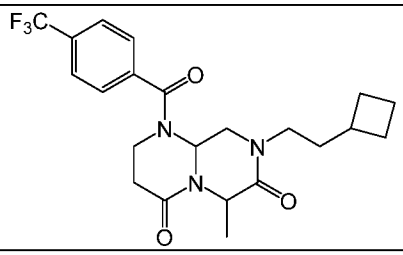
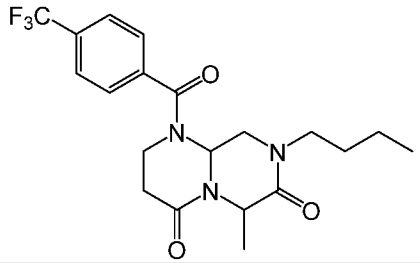
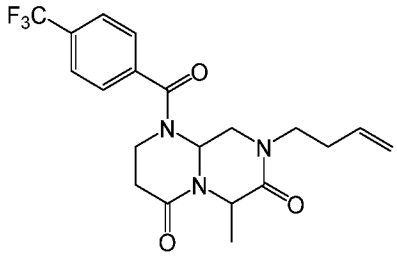
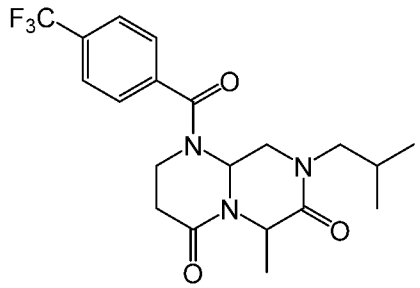
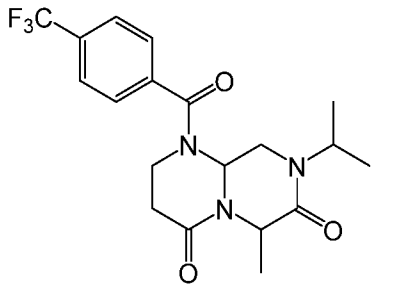
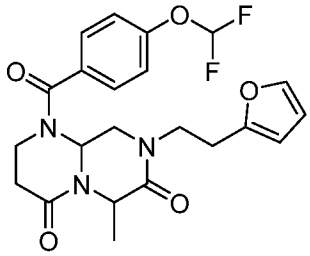
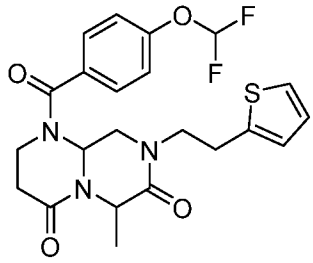
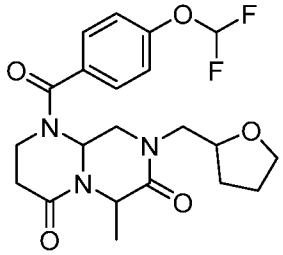
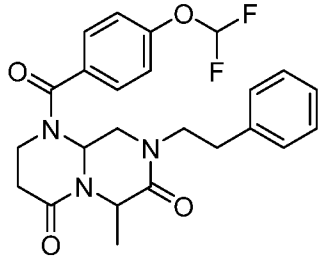
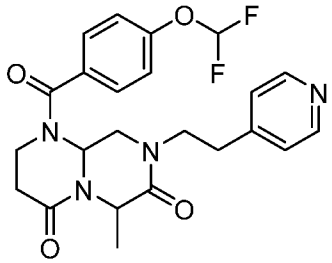
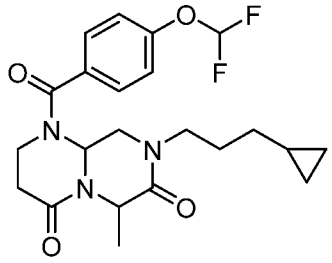
[0099] In some embodiments, provided is a compound selected from the compounds in Table 1 or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. Although certain compounds described in the present disclosure, including in Table 1, are presented as specific stereoisomers and/or in a non-stereochemical form, it is understood that any or all stereochemical forms, including any enantiomeric or diastereomeric forms, and any tautomers or other forms of any of the compounds of the present disclosure, including in Table 1, are herein described.

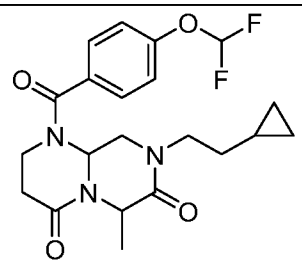
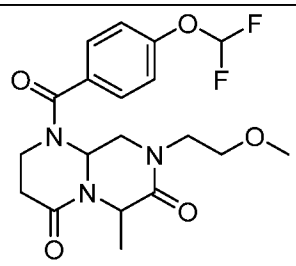
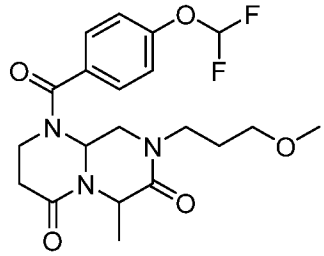
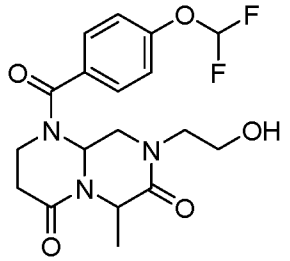
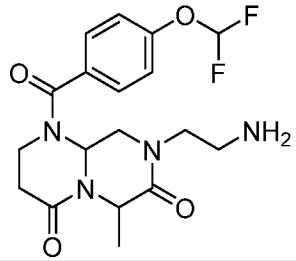
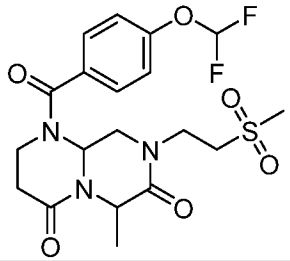
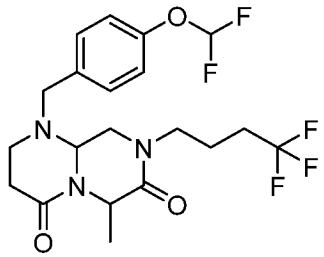
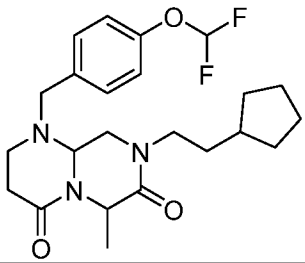
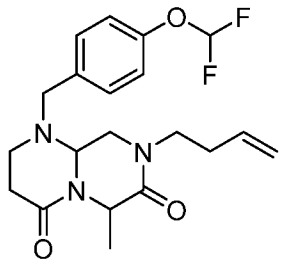
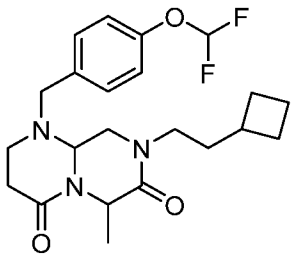
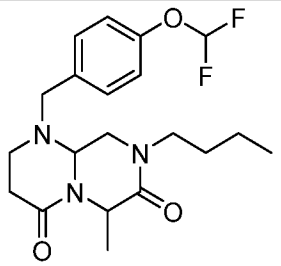
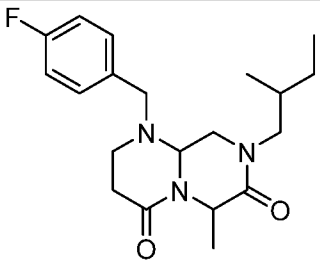
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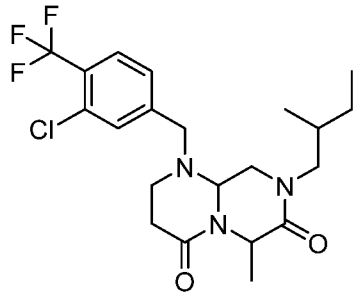
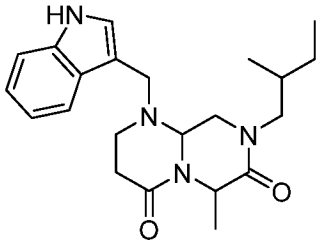
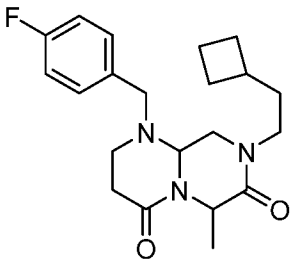
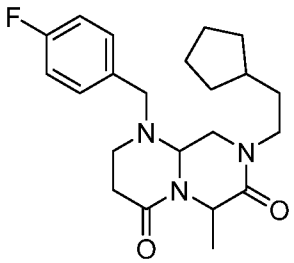
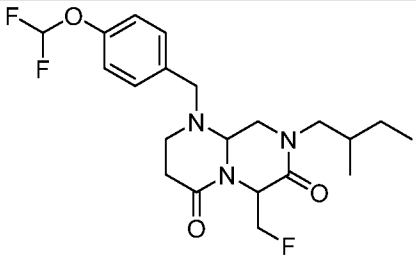
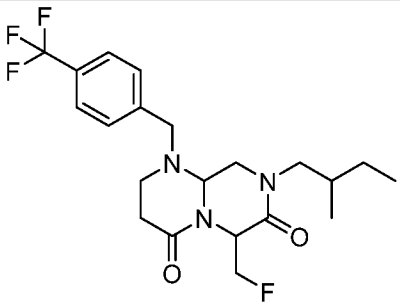
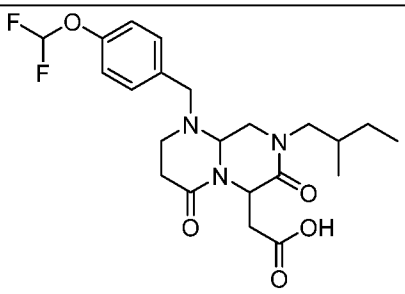
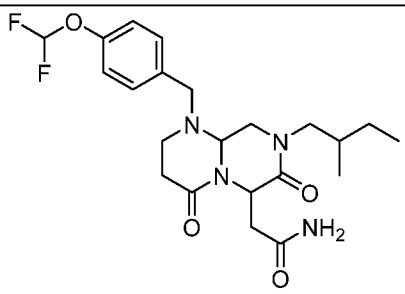
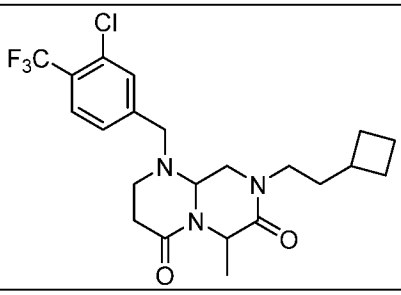
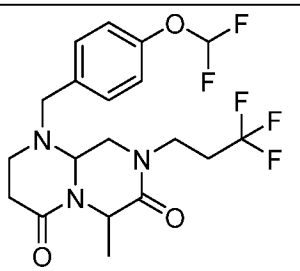
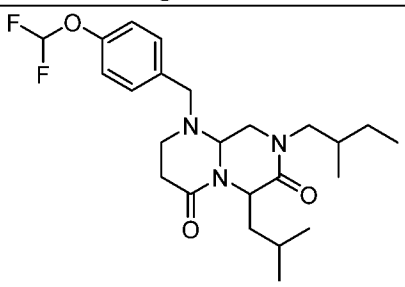
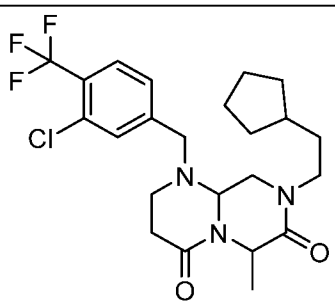
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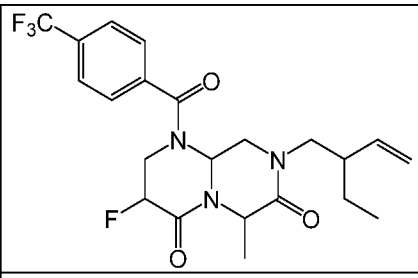
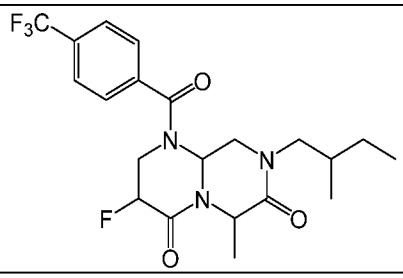
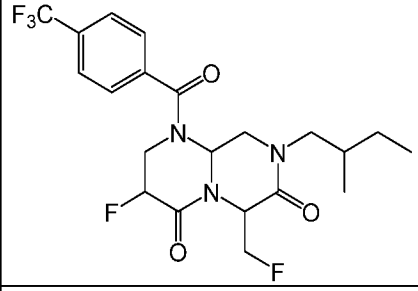
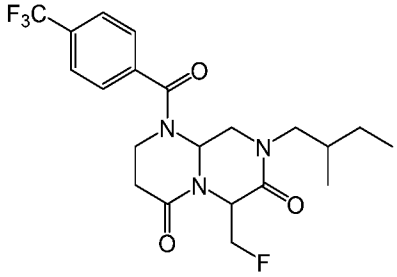
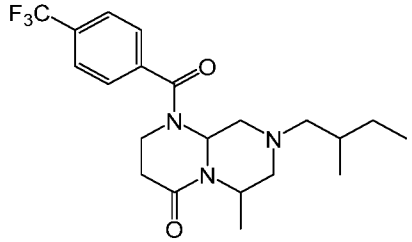
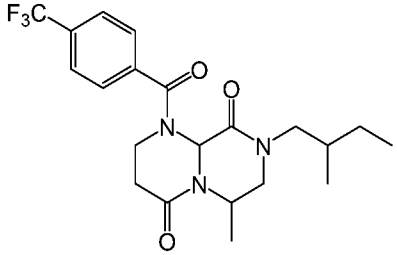
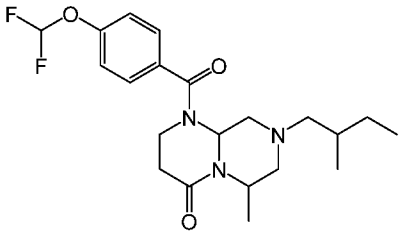
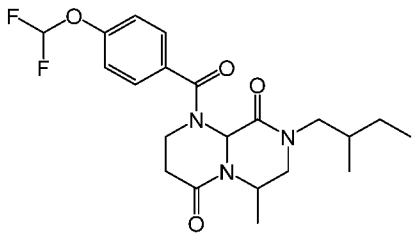
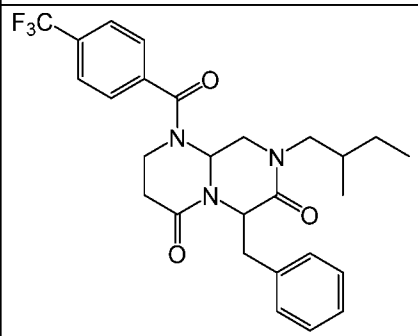
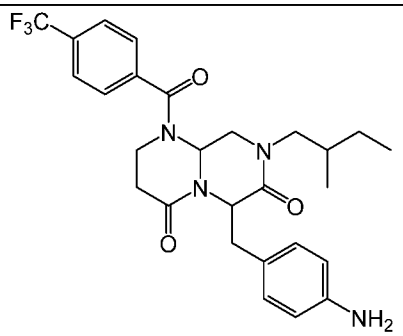
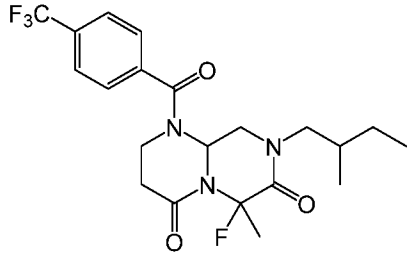
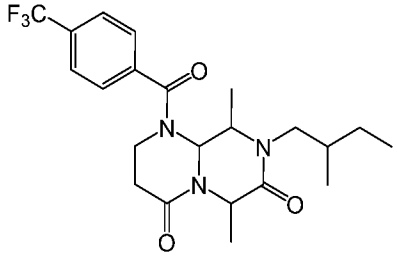
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5a		**5b**	
6a		**6b**	
7a		**7b**	

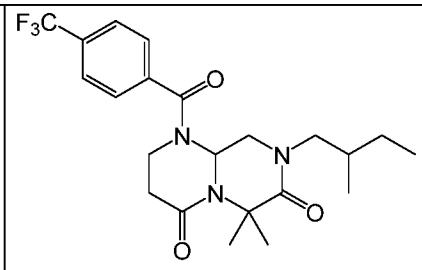
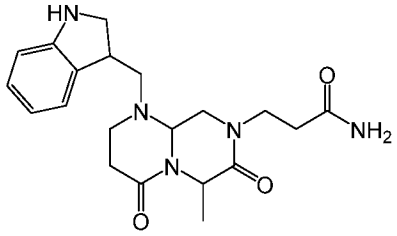
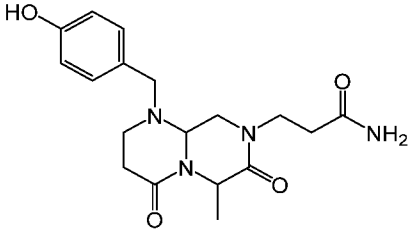
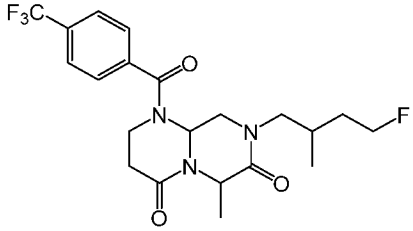
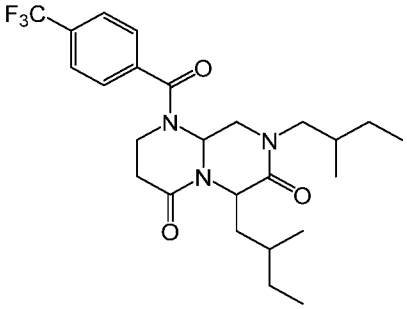
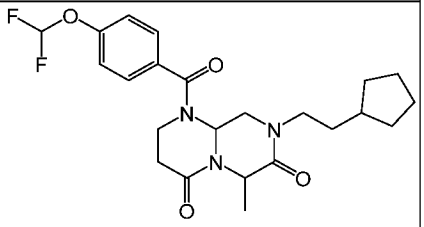
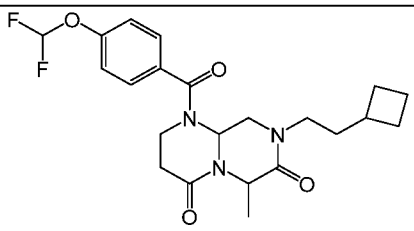
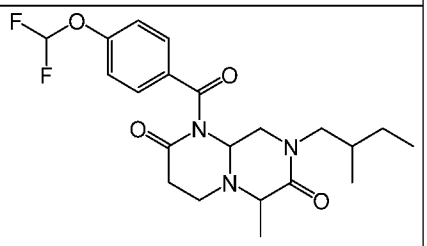
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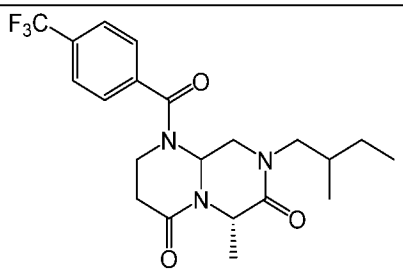
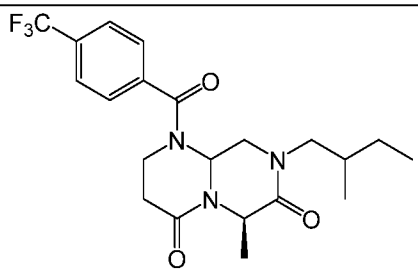
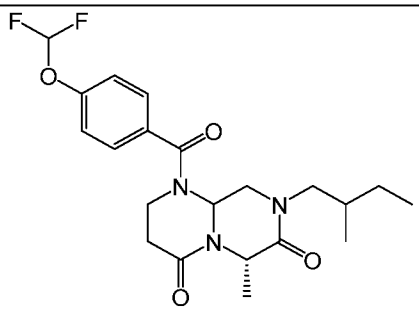
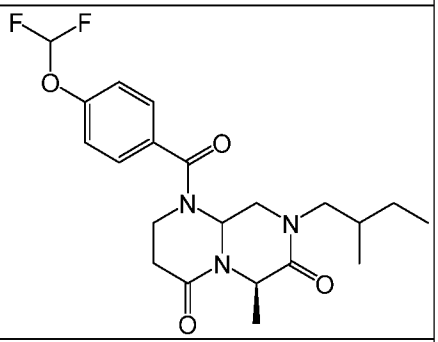
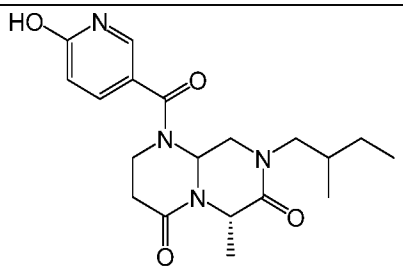
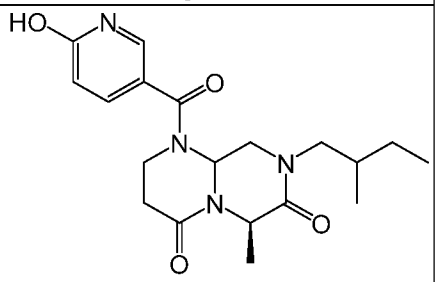
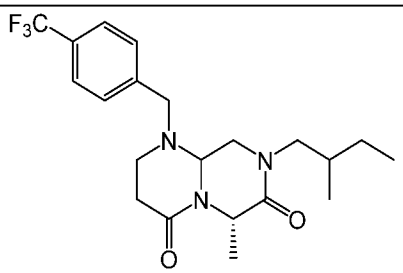
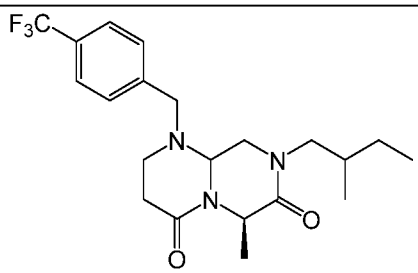
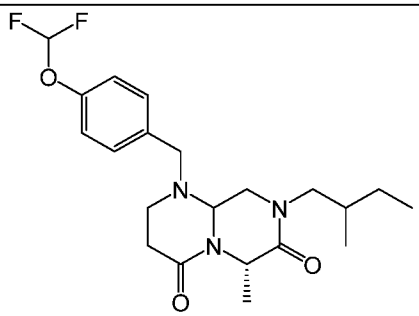
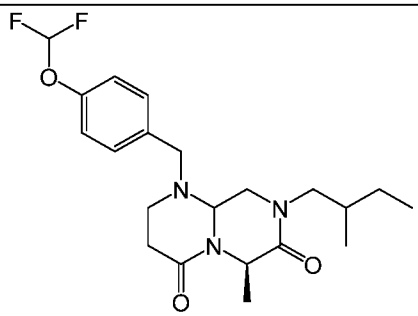
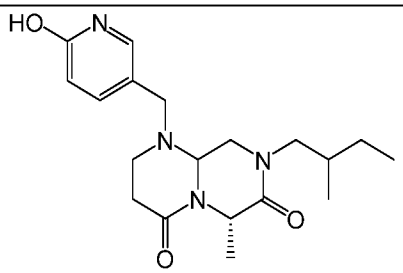
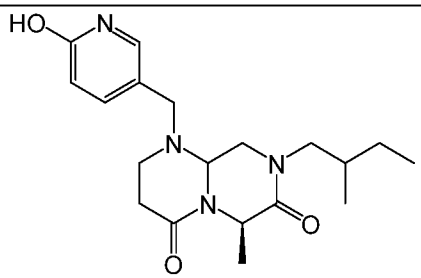
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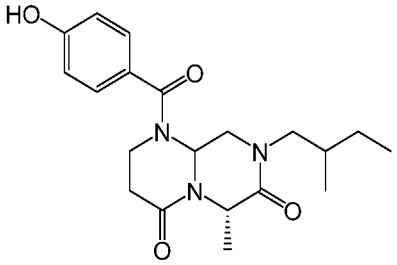
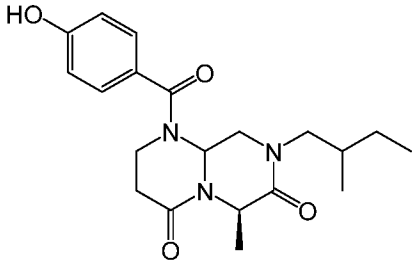
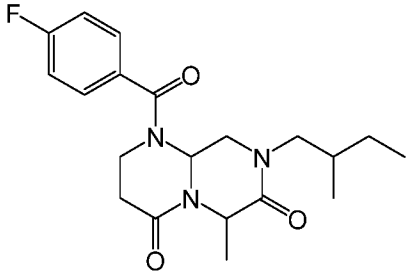
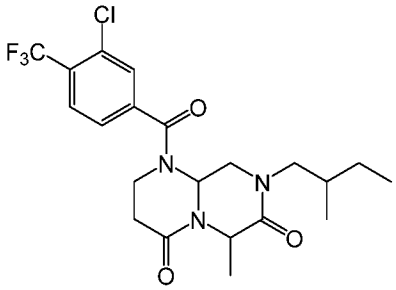
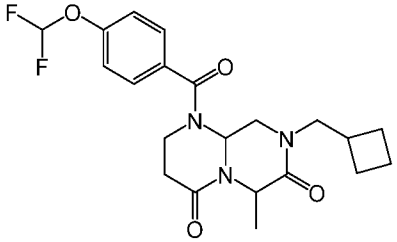
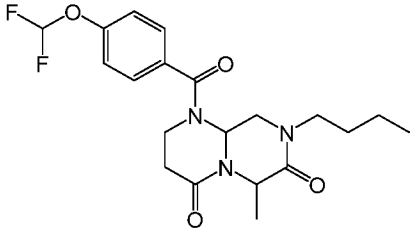
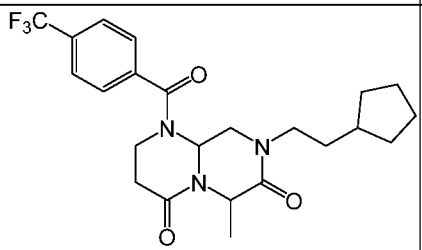
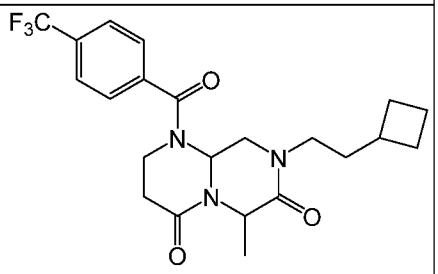
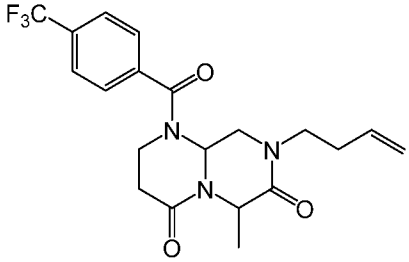
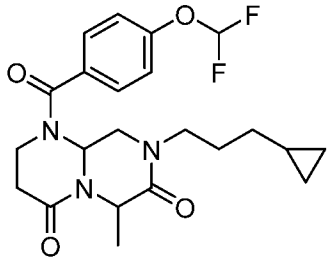
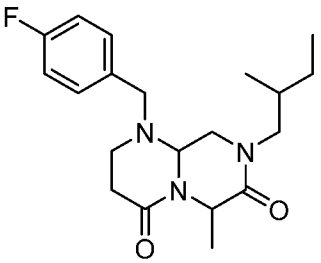
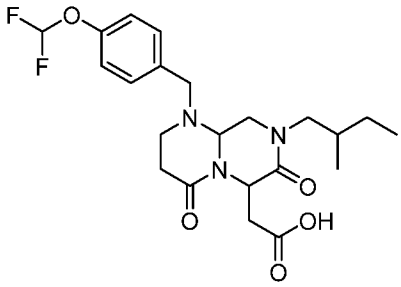
or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof.

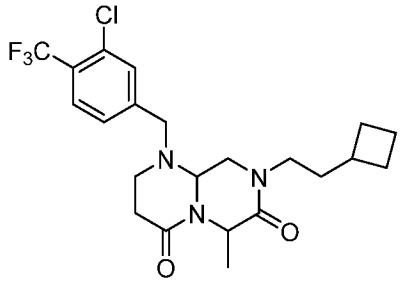
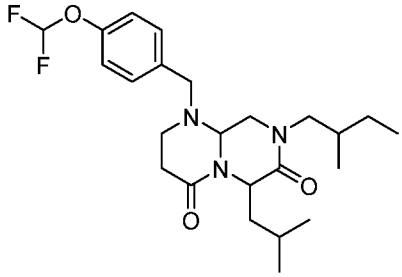
[0100] In some embodiments, the compound of Formula (I) is not Compound **3a**, **3b**, **9**, **10**, **13**, **15**, **16**, **18**, **21**, **23-29**, **31-41**, **43-48**, **50**, **52**, or **54**.

[0101] In some embodiments, provided is a compound selected from the compounds in Table 1A or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. Although certain compounds described in the present disclosure, including in Table 1A, are presented as specific stereoisomers and/or in a non-stereochemical form, it is understood that any or all stereochemical forms, including any enantiomeric or diastereomeric forms, and any tautomers or other forms of any of the compounds of the present disclosure, including in Table 1A, are herein described.

Table 1A.

Compound	Structure	Compound	Structure
1a		**1b**	
2a		**2b**	
4a		**4b**	
5a		**5b**	
6a		**6b**	
7a		**7b**	

8a		**8b**	
11		**12**	
14		**17**	
19		**20**	
22		**30**	
42		**49**	

51		53	

or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof.

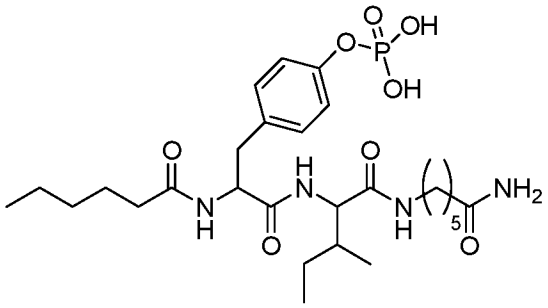
[0102] It is understood that in the present description, combinations of substituents and/or variables of the depicted formulae are permissible only if such contributions result in stable compounds.

[0103] Furthermore, all compounds of Formula (I) which exist in free base or acid form can be converted to their pharmaceutically acceptable salts by treatment with the appropriate inorganic or organic base or acid by methods known to one skilled in the art. Salts of the compounds of Formula (I) can be converted to their free base or acid form by standard techniques.

Fosgonimeton and related compounds

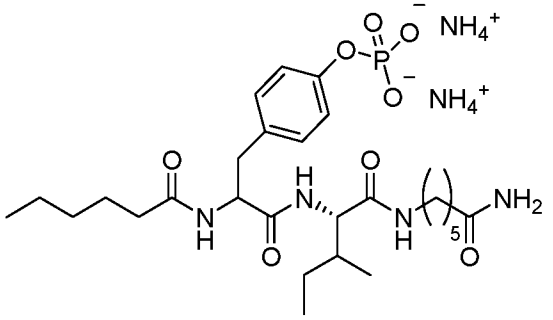
[0104] Fosgonimeton is a prodrug that is rapidly converted to the active drug ATH-1001 (Dihexa; see US2014/0094413) in the plasma after subcutaneous injection. The active drug ATH-1001 acts as a positive modulator of the hepatic growth factor (HGF) receptor and its tyrosine kinase, MET, receptor system.

[0105] Fosgonimeton is a pharmaceutically acceptable salt of the compound A19:

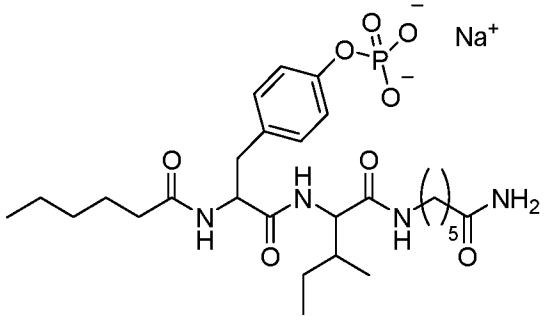


A19

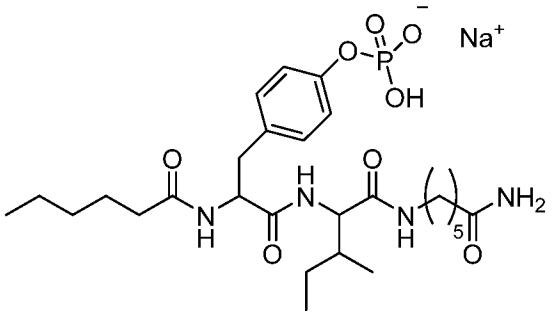
[0106] Nonlimiting exemplary pharmaceutically acceptable salts of compound A19 include:



,

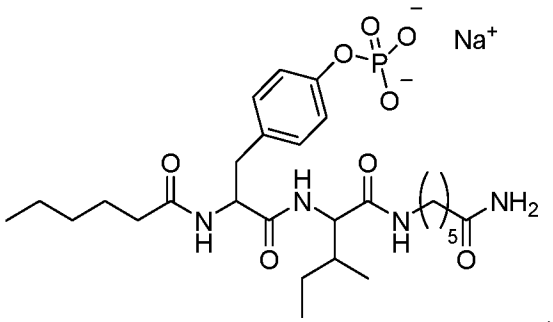


, and



.

[0107] Unless otherwise indicated, fosgonimeton refers to the monosodium salt of compound A19, shown below:



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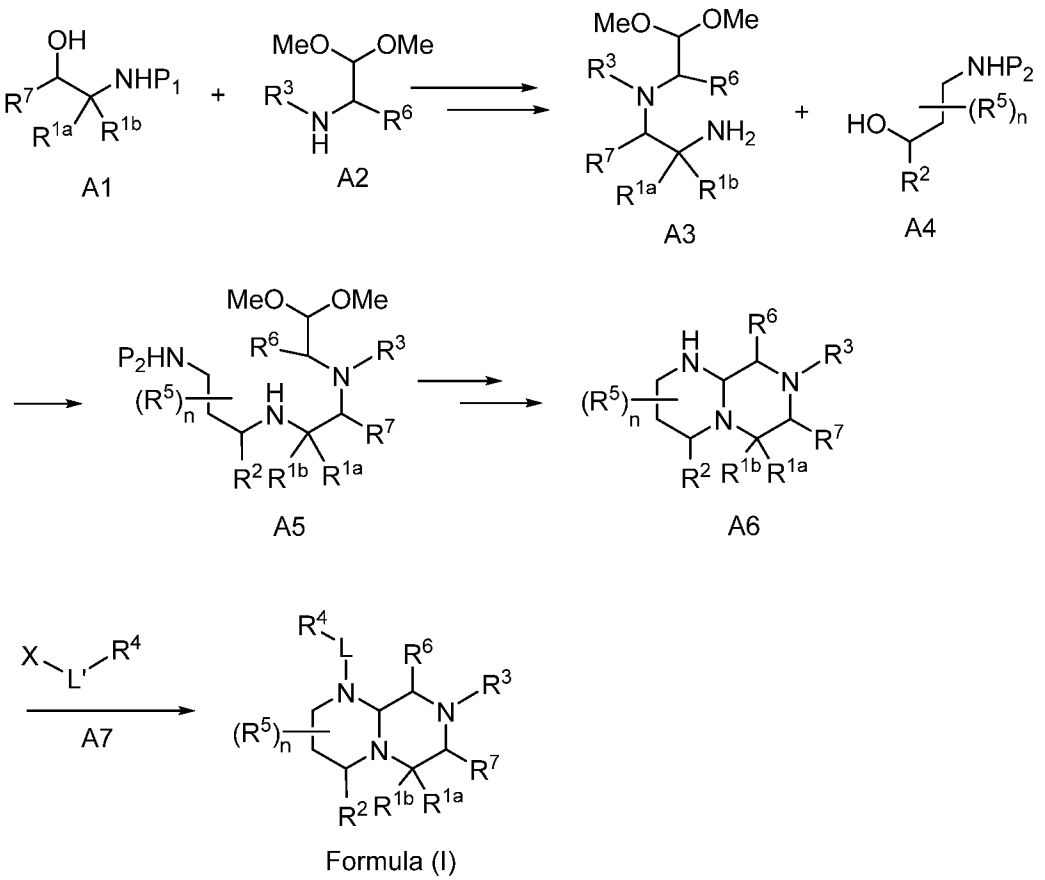
[0108] The compound A19, and pharmaceutically acceptable salts, isotopic forms, and stereoisomers thereof, including fosgonimeton, may be synthesized and characterized using methods known to those of skill in the art, such as those described in PCT Publication No. WO 2017/210489 A1.

[0109] In some embodiments, fosgonimeton is formulated for subcutaneous administration.

Methods of Synthesis

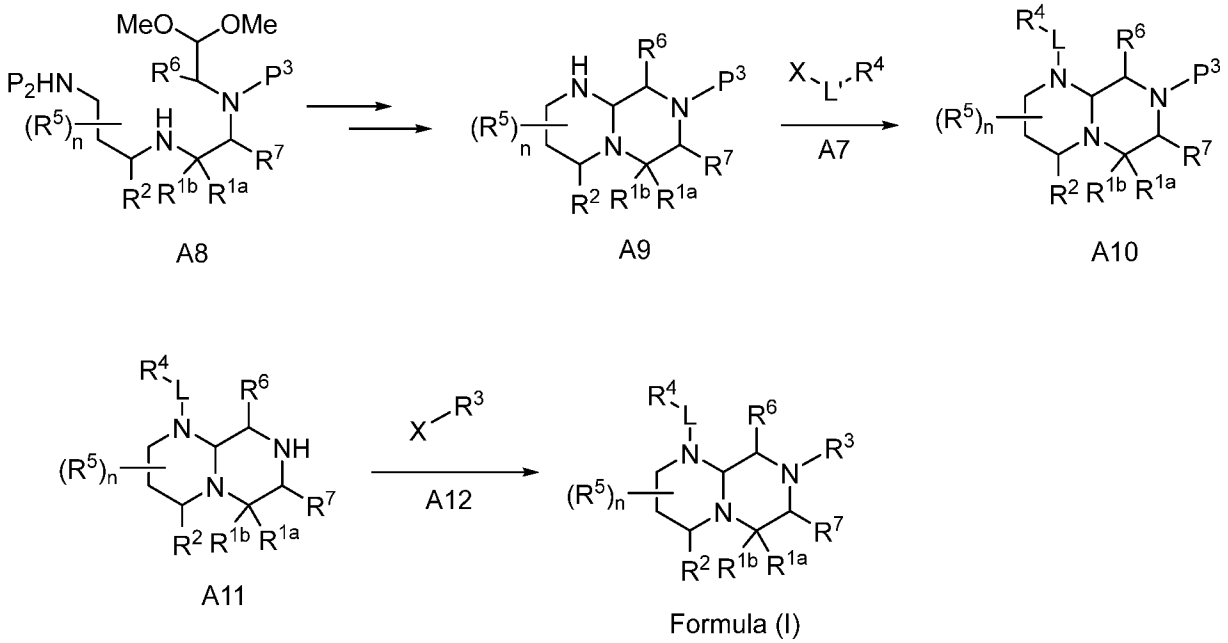
[0110] Compounds of Formula (I), or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, can be prepared by using organic chemistry synthesis methods known in the art. In general, starting components may be obtained from sources such as Sigma Aldrich, Lancaster Synthesis, Inc., Maybridge, Matrix Scientific, TCI, and Fluorochem USA, etc. or synthesized according to sources known to those skilled in the art (see, for example, Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th edition (Wiley, December 2000)) or prepared as described herein.

General Reaction Scheme 1.

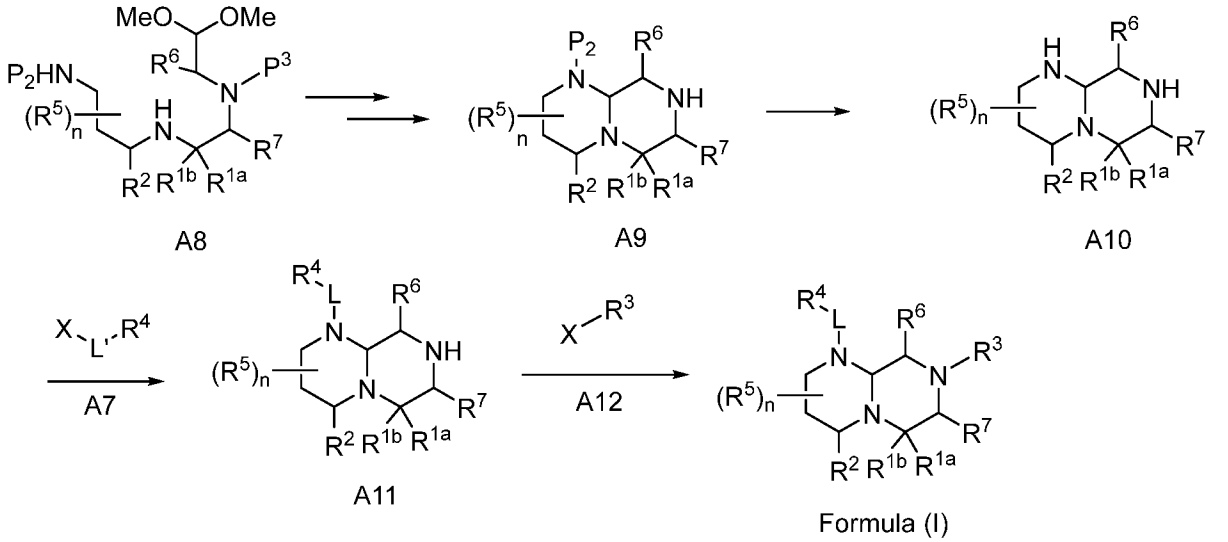


[0111] General Reaction Scheme 1 provides an exemplary method for preparation of compounds of Formula (I). R^{1a}, R^{1b}, R^2, R^3, R^4, R^5, R^6, R^7, L, and n in General Reaction Scheme 1 are as defined herein. X is a reactive moiety selected to facilitate the desired reaction (*e.g.*, halo). P_1 and P_2 are suitable protecting groups. L' is selected such that a desired L moiety results from the reaction between L'-R^4 and the secondary amine. Compounds of structure A1 are purchased or prepared according to methods known in the art. Reaction of A1 with A2 under appropriate coupling conditions (*e.g.*, T_3P and base) yields the product of the coupling reaction between A1 and A2, A3. A3 is then reacted with A4 under suitable coupling conditions (*e.g.*, T_3P and base) to afford compound A5. Compound A5 is then cyclized (*e.g.*, using formic acid) and deprotected (*e.g.*, using piperidine) to afford compound A6. Compound A6 is then reacted with compound A7 to afford the final compound of Formula (I) as shown.

General Reaction Scheme 2.



[0112] An alternative method for the synthesis of compounds of Formula (I) is depicted in General Reaction Scheme 2. R^{1a}, R^{1b}, R^2, R^3, R^4, R^5, R^6, R^7, L, and n in General Reaction Scheme 2 are as defined herein. P_2 is a suitable protecting group. Each X is a reactive moiety selected to facilitate the desired reaction (*e.g.*, halo). L' is selected such that a desired L moiety results from the reaction between L'-R^4 and the secondary amine. Intermediate A5 is prepared with a removable protecting group P^3 (e.g. para-methoxybenzyl) as the R^3 group giving intermediate A8. A8 is then cyclized (*e.g.*, using formic acid) and deprotected (*e.g.*, using piperidine) to afford compound A9. Compound A9 is then reacted with A7 to give compound A10. Compound A10 is then deprotected (*e.g.*, with cerica ammonium nitrate) to give compound A11. Compound A11 is then reacted with A12 to provide the final compound of Formula (I). General Reaction Scheme 3.



[0113] A related method to the one shown in General Reaction Scheme 2 is depicted in General Reaction Scheme 3. In this method, the two amine nitrogen atoms of the bicyclic core

are deprotected to provide compound A10, then reacted with A7 to afford compound A11. Subsequent reaction with A12 provides the final compound of Formula (I).

[0114] It should be noted that various alternative strategies for preparation of compounds of Formula (I) are available to those of ordinary skill in the art. For example, other compounds of Formula (I) can be prepared according to analogous methods using the appropriate starting material.

[0115] It will also be appreciated by those skilled in the art that in the processes for preparing the compounds described herein the functional groups of intermediate compounds may need to be protected by suitable protecting groups. Such functional groups may include hydroxy, amino, and carboxylic acid. Suitable protecting groups for hydroxy include trialkylsilyl or diarylalkylsilyl (for example, *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl or trimethylsilyl), tetrahydropyranyl, benzyl, and the like. Suitable protecting groups for amino and amidino include *t*-butoxycarbonyl, benzyloxycarbonyl, and the like. Suitable protecting groups for carboxylic acid include alkyl, aryl, or arylalkyl esters. Protecting groups are optionally added or removed in accordance with standard techniques, which are known to one skilled in the art and as described herein. The use of protecting groups is described in detail in Green, T.W. and P.G.M. Wutz, *Protective Groups in Organic Synthesis* (1999), 3rd Ed., Wiley. As one of skill in the art would appreciate, the protecting group may also be a polymer resin such as a Wang resin, Rink resin or a 2-chlorotrityl-chloride resin.

Pharmaceutical Compositions and Formulations

[0116] In a further aspect, provided herein are pharmaceutical compositions. The pharmaceutical composition comprises any one (or more) of the foregoing compounds and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition is formulated for oral administration. In other embodiments, the pharmaceutical composition is formulated for injection. In still more embodiments, the pharmaceutical compositions comprise a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, and an additional therapeutic agent. Non-limiting examples of such therapeutic agents are described herein below.

[0117] Suitable routes of administration include, but are not limited to, oral, intravenous, rectal, aerosol, parenteral, ophthalmic, pulmonary, transmucosal, transdermal, vaginal, otic, nasal, and topical administration. In addition, by way of example only, parenteral delivery includes intramuscular, subcutaneous, intravenous, intramedullary injections, as well as intrathecal, direct intraventricular, intraperitoneal, intralymphatic, and intranasal injections.

[0118] In certain embodiments, a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, is administered in a local rather than systemic manner, for example, via injection of the compound directly into an organ, often in a depot preparation or sustained release formulation. In specific embodiments, long acting formulations are administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Furthermore, in other embodiments, the drug is delivered in a targeted drug delivery system, for example, in a liposome coated with organ-specific antibody. In such embodiments, the liposomes are targeted to and taken up selectively by the organ. In yet other embodiments, the compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, is provided in the form of a rapid release formulation, in the form of an extended release formulation, or in the form of an intermediate release formulation. In yet other embodiments, the compound described herein is administered topically.

[0119] The compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are effective over a wide dosage range. For example, in the treatment of adult humans, dosages from 0.01 to 1000 mg, from 0.5 to 100 mg, from 1 to 50 mg per day, and from 5 to 40 mg per day are examples of dosages that are used in some embodiments. An exemplary dosage is 10 to 30 mg per day. The exact dosage will depend upon the route of administration, the form in which the compound is administered, the subject to be treated, the body weight of the subject to be treated, and the preference and experience of the attending physician.

[0120] In some embodiments, a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, is administered in a single dose. Typically, such administration will be by injection, *e.g.*, intravenous injection, in order to introduce the agent quickly. However, other routes are used as appropriate. A single dose of a compound of the disclosure may also be used for treatment of an acute condition.

[0121] In some embodiments, a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, is administered in multiple doses. In some embodiments, dosing is about once, twice, three times, four times, five times, six times, or more than six times per day. In other embodiments, dosing is about once a month, once every two weeks, once a week, or once every other day. In another embodiment a compound of Formula (I), or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, and another therapeutic agent are administered together about once per day to about 6 times per day. In another embodiment the administration of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof,

and a therapeutic agent continues for less than about 7 days. In yet another embodiment the administration continues for more than about 6, 10, 14, 28 days, two months, six months, or one year. In some cases, continuous dosing is achieved and maintained as long as necessary.

[0122] Administration of the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, may continue as long as necessary. In some embodiments, a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, is administered for more than 1, 2, 3, 4, 5, 6, 7, 14, or 28 days. In some embodiments, a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, is administered for less than 28, 14, 7, 6, 5, 4, 3, 2, or 1 day. In some embodiments, a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, is administered chronically on an ongoing basis, *e.g.*, for the treatment of chronic effects (*e.g.*, neuroinflammatory conditions).

[0123] In some embodiments, the compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, is administered in dosages. It is known in the art that due to intersubject variability in compound pharmacokinetics, individualization of dosing regimen is necessary for optimal therapy. Dosing for a compound may be found by routine experimentation in light of the instant disclosure.

[0124] In some embodiments, the compounds Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are formulated into pharmaceutical compositions. In specific embodiments, pharmaceutical compositions are formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Any pharmaceutically acceptable techniques, carriers, and excipients are used as suitable to formulate the pharmaceutical compositions described herein: Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980; and Pharmaceutical Dosage Forms and Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins1999).

[0125] Provided herein are pharmaceutical compositions comprising a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, and a pharmaceutically acceptable diluent(s), excipient(s), or carrier(s). Also provided herein are methods for administering a pharmaceutical composition comprising a

compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, and a pharmaceutically acceptable diluent(s), excipient(s), or carrier(s).

[0126] In certain embodiments, the compounds are administered as pharmaceutical compositions in which compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are mixed with other therapeutic agents, as in combination therapy. Encompassed herein are all combinations of active ingredients set forth in the methods section below and throughout this disclosure. In specific embodiments, the pharmaceutical compositions include one or more compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof.

[0127] A pharmaceutical composition, as used herein, refers to a mixture of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, with other chemical components, such as carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, and/or excipients. In certain embodiments, the pharmaceutical composition facilitates administration of the compound to an organism. In some embodiments, practicing the methods of treatment or use provided herein, therapeutically effective amounts of compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, provided herein are administered in a pharmaceutical composition to a mammal having a disease, disorder or medical condition to be treated. In specific embodiments, the mammal is a human. In certain embodiments, therapeutically effective amounts vary depending on the severity of the disease, the age and relative health of the subject, the potency of the compound used and other factors. The compounds described herein are used singly or in combination with one or more therapeutic agents as components of mixtures.

[0128] In one embodiment, one or more compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are formulated in an aqueous solution. In specific embodiments, the aqueous solution is selected from, by way of example only, a physiologically compatible buffer, such as Hank's solution, Ringer's solution, or physiological saline buffer. In other embodiments, one or more compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are formulated for transmucosal administration. In specific embodiments, transmucosal formulations include penetrants that are appropriate to the barrier to be permeated (*e.g.*, the blood-brain barrier). In still other embodiments wherein the compounds described herein are formulated for other parenteral injections, appropriate formulations include aqueous or non-

aqueous solutions. In specific embodiments, such solutions include physiologically compatible buffers and/or excipients.

[0129] In another embodiment, compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are formulated for oral administration. Compounds are formulated by combining the active compounds with, *e.g.*, pharmaceutically acceptable carriers or excipients. In various embodiments, the compounds Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are formulated in oral dosage forms that include, by way of example only, tablets, powders, pills, dragees, capsules, liquids, gels, syrups, elixirs, slurries, suspensions, and the like.

[0130] In certain embodiments, pharmaceutical preparations for oral use are obtained by mixing one or more solid excipients with one or more of the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as: for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methylcellulose, microcrystalline cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose; or others such as: polyvinylpyrrolidone (PVP or povidone) or calcium phosphate. In specific embodiments, disintegrating agents are optionally added. Disintegrating agents include, by way of example only, cross-linked croscarmellose sodium, polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0131] In one embodiment, dosage forms, such as dragee cores and tablets, are provided with one or more suitable coating. In specific embodiments, concentrated sugar solutions are used for coating the dosage form. The sugar solutions, optionally contain additional components, such as by way of example only, gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs and/or pigments are also optionally added to the coatings for identification purposes. Additionally, the dyestuffs and/or pigments are optionally utilized to characterize different combinations of active compound doses.

[0132] In certain embodiments, therapeutically effective amounts of at least one of the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are formulated into other oral dosage forms. Oral dosage forms include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. In specific embodiments, push-fit capsules contain the

active ingredients in admixture with one or more fillers. Fillers include, by way of example only, lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In other embodiments, soft capsules contain one or more active compound that is/are dissolved or suspended in a suitable liquid. Suitable liquids include, by way of example only, one or more fatty oil, liquid paraffin, or liquid polyethylene glycol. In addition, stabilizers are optionally added.

[0133] In other embodiments, therapeutically effective amounts of at least one of the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, described herein are formulated for buccal or sublingual administration. Formulations suitable for buccal or sublingual administration include, by way of example only, tablets, lozenges, or gels. In still other embodiments, the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are formulated for parental injection, including formulations suitable for bolus injection or continuous infusion. In specific embodiments, formulations for injection are presented in unit dosage forms (*e.g.*, in ampoules) or in multi-dose containers. Preservatives are, optionally, added to the injection formulations. In still other embodiments, the pharmaceutical compositions are formulated in a form suitable for parenteral injection as sterile suspensions, solutions or emulsions in oily or aqueous vehicles. Parenteral injection formulations optionally contain formulatory agents such as suspending, stabilizing and/or dispersing agents. In specific embodiments, pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. In additional embodiments, a suspension of an active compound or compounds (*e.g.*, compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof,) are prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles for use in the pharmaceutical compositions described herein include, by way of example only, fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. In certain specific embodiments, aqueous injection suspensions contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension contains suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, in other embodiments, the active ingredient is in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[0134] In still other embodiments, the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are administered topically. The compounds are formulated into a variety of topically administrable compositions,

such as solutions, suspensions, lotions, gels, pastes, medicated sticks, balms, creams or ointments. Such pharmaceutical compositions optionally contain solubilizers, stabilizers, tonicity enhancing agents, buffers and preservatives.

[0135] In yet other embodiments, the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are formulated for transdermal administration. In specific embodiments, transdermal formulations employ transdermal delivery devices and transdermal delivery patches and can be lipophilic emulsions or buffered, aqueous solutions, dissolved and/or dispersed in a polymer or an adhesive. In various embodiments, such patches are constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents. In additional embodiments, the transdermal delivery of the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, is accomplished by means of iontophoretic patches and the like. In certain embodiments, transdermal patches provide controlled delivery of the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In specific embodiments, the rate of absorption is slowed by using rate-controlling membranes or by trapping the compound within a polymer matrix or gel. In alternative embodiments, absorption enhancers are used to increase absorption. Absorption enhancers or carriers include absorbable pharmaceutically acceptable solvents that assist passage through the skin. For example, in one embodiment, transdermal devices are in the form of a bandage comprising a backing member, a reservoir containing the compound optionally with carriers, optionally a rate controlling barrier to deliver the compound to the skin of the host at a controlled and predetermined rate over a prolonged period of time, and means to secure the device to the skin.

[0136] In other embodiments, the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are formulated for administration by inhalation. Various forms suitable for administration by inhalation include, but are not limited to, aerosols, mists or powders. Pharmaceutical compositions of any of the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant (*e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In specific embodiments, the dosage unit of a pressurized aerosol is determined by providing a valve to deliver a metered amount. In certain embodiments, capsules and cartridges of, such as, by way of example only, gelatin for use in an inhaler or insufflator is

formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0137] In still other embodiments, the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are formulated in rectal compositions such as enemas, rectal gels, rectal foams, rectal aerosols, suppositories, jelly suppositories, or retention enemas, containing conventional suppository bases such as cocoa butter or other glycerides, as well as synthetic polymers such as polyvinylpyrrolidone, PEG, and the like. In suppository forms of the compositions, a low-melting wax such as, but not limited to, a mixture of fatty acid glycerides, optionally in combination with cocoa butter is first melted.

[0138] In certain embodiments, pharmaceutical compositions are formulated in any conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Any pharmaceutically acceptable techniques, carriers, and excipients are optionally used as suitable. Pharmaceutical compositions comprising a compound of Formula (I), or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are manufactured in a conventional manner, such as, by way of example only, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or compression processes.

[0139] Pharmaceutical compositions include at least one pharmaceutically acceptable carrier, diluent or excipient and at least one compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, described herein as an active ingredient. The active ingredient is in free-acid or free-base form, or in a pharmaceutically acceptable salt form. In addition, the methods and pharmaceutical compositions described herein include the use of N-oxides, crystalline forms (also known as polymorphs), as well as active metabolites of these compounds having the same type of activity. All tautomers of the compounds described herein are included within the scope of the compounds presented herein. Additionally, the compounds Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, encompass unsolvated as well as solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like. The solvated forms of the compounds presented herein are also considered to be disclosed herein. In addition, the pharmaceutical compositions optionally include other medicinal or pharmaceutical agents, carriers, adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure, buffers, and/or other therapeutically valuable substances.

[0140] Methods for the preparation of compositions comprising the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, include formulating the compounds with one or more inert, pharmaceutically acceptable excipients or carriers to form a solid, semi-solid or liquid. Solid compositions include, but are not limited to, powders, tablets, dispersible granules, capsules, cachets, and suppositories. Liquid compositions include solutions in which a compound is dissolved, emulsions comprising a compound, or a solution containing liposomes, micelles, or nanoparticles comprising a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. Semi-solid compositions include, but are not limited to, gels, suspensions and creams. The form of the pharmaceutical compositions described herein include liquid solutions or suspensions, solid forms suitable for solution or suspension in a liquid prior to use, or as emulsions. These compositions also optionally contain minor amounts of nontoxic, auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, and so forth.

[0141] In some embodiments, pharmaceutical composition comprising at least one compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, illustratively takes the form of a liquid where the agents are present in solution, in suspension or both. Typically, when the composition is administered as a solution or suspension a first portion of the agent is present in solution and a second portion of the agent is present in particulate form, in suspension in a liquid matrix. In some embodiments, a liquid composition includes a gel formulation. In other embodiments, the liquid composition is aqueous.

[0142] In certain embodiments, useful aqueous suspensions contain one or more polymers as suspending agents. Useful polymers include water-soluble polymers such as cellulosic polymers, *e.g.*, hydroxypropyl methylcellulose, and water-insoluble polymers such as cross-linked carboxyl-containing polymers. Certain pharmaceutical compositions described herein comprise a mucoadhesive polymer, selected for example from carboxymethylcellulose, carbomer (acrylic acid polymer), poly(methylmethacrylate), polyacrylamide, polycarbophil, acrylic acid/butyl acrylate copolymer, sodium alginate and dextran.

[0143] Useful pharmaceutical compositions also, optionally, include solubilizing agents to aid in the solubility of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. The term "solubilizing agent" generally includes agents that result in formation of a micellar solution or a true solution of the agent. Certain acceptable nonionic surfactants, for example polysorbate 80, are useful as solubilizing agents, as can ophthalmically acceptable glycols, polyglycols, *e.g.*, polyethylene glycol 400, and glycol ethers.

[0144] Furthermore, useful pharmaceutical compositions optionally include one or more pH adjusting agents or buffering agents, including acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-hydroxymethylaminomethane; and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an acceptable range.

[0145] Additionally, useful compositions also, optionally, include one or more salts in an amount required to bring osmolality of the composition into an acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate, or bisulfite anions; suitable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite, and ammonium sulfate.

[0146] Other useful pharmaceutical compositions optionally include one or more preservatives to inhibit microbial activity. Suitable preservatives include mercury-containing substances such as merfen and thiomersal; stabilized chlorine dioxide; and quaternary ammonium compounds such as benzalkonium chloride, cetyltrimethylammonium bromide, and cetylpyridinium chloride.

[0147] Still other useful compositions include one or more surfactants to enhance physical stability or for other purposes. Suitable nonionic surfactants include polyoxyethylene fatty acid glycerides and vegetable oils, *e.g.*, polyoxyethylene (60) hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, *e.g.*, octoxynol 10 and octoxynol 40.

[0148] Still other useful compositions include one or more antioxidants to enhance chemical stability where required. Suitable antioxidants include, by way of example only, ascorbic acid and sodium metabisulfite.

[0149] In certain embodiments, aqueous suspension compositions are packaged in single-dose non-reclosable containers. Alternatively, multiple-dose reclosable containers are used, in which case it is typical to include a preservative in the composition.

[0150] In alternative embodiments, other delivery systems for hydrophobic pharmaceutical compounds are employed. Liposomes and emulsions are examples of delivery vehicles or carriers useful herein. In certain embodiments, organic solvents such as N-methylpyrrolidone are also employed. In additional embodiments, the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials are useful herein.

In some embodiments, sustained-release capsules release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization are employed.

[0151] In certain embodiments, the formulations described herein comprise one or more antioxidants, metal chelating agents, thiol containing compounds and/or other general stabilizing agents. Examples of such stabilizing agents, include, but are not limited to: (a) about 0.5% to about 2% w/v glycerol, (b) about 0.1% to about 1% w/v methionine, (c) about 0.1% to about 2% w/v monothioglycerol, (d) about 1 mM to about 10 mM EDTA, (e) about 0.01% to about 2% w/v ascorbic acid, (f) 0.003% to about 0.02% w/v polysorbate 80, (g) 0.001% to about 0.05% w/v. polysorbate 20, (h) arginine, (i) heparin, (j) dextran sulfate, (k) cyclodextrins, (l) pentosan polysulfate and other heparinoids, (m) divalent cations such as magnesium and zinc; or (n) combinations thereof.

[0152] In some embodiments, the concentration of the compound of Formula (I) or compound A19 provided in the pharmaceutical compositions of the present disclosure is less than 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 19%, 18%, 17%, 16%, 15%,14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.09%, 0.08%, 0.07%, 0.06%, 0.05%, 0.04%, 0.03%, 0.02%, 0.01%, 0.009%, 0.008%, 0.007%, 0.006%, 0.005%, 0.004%, 0.003%, 0.002%, 0.001%, 0.0009%, 0.0008%, 0.0007%, 0.0006%, 0.0005%, 0.0004%, 0.0003%, 0.0002%, or 0.0001% w/w, w/v or v/v.

[0153] In some embodiments, the concentration of the compound of Formula (I) or compound A19 provided in the pharmaceutical compositions of the present disclosure is greater than 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 19.75%, 19.50%, 19.25%, 19%, 18.75%, 18.50%, 18.25%, 18%, 17.75%, 17.50%, 17.25%, 17%, 16.75%, 16.50%, 16.25%, 16%, 15.75%, 15.50%, 15.25%, 15%, 14.75%, 14.50%, 14.25%, 14%, 13.75%, 13.50%, 13.25%, 13%, 12.75%, 12.50%, 12.25%, 12%, 11.75%, 11.50%, 11.25%, 11%, 10.75%, 10.50%, 10.25%, 10%, 9.75%, 9.50%, 9.25%, 9%, 8.75%, 8.50%, 8.25%, 8%, 7.75%, 7.50%, 7.25%, 7%, 6.75%, 6.50%, 6.25%, 6%, 5.75%, 5.50%, 5.25%, 5%, 4.75%, 4.50%, 4.25%, 4%, 3.75%, 3.50%, 3.25%, 3%, 2.75%, 2.50%, 2.25%, 2%, 1.75%, 1.50%, 125% , 1%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.09%, 0.08%, 0.07%, 0.06%, 0.05%, 0.04%, 0.03%, 0.02%, 0.01%, 0.009%, 0.008%, 0.007%, 0.006%, 0.005%, 0.004%, 0.003%, 0.002%, 0.001%, 0.0009%, 0.0008%, 0.0007%, 0.0006%, 0.0005%, 0.0004%, 0.0003%, 0.0002%, or 0.0001% w/w, w/v, or v/v.

[0154] In some embodiments, the concentration of the compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, provided in the pharmaceutical compositions ranges from approximately 0.0001% to approximately 50%, approximately 0.001% to approximately 40 %, approximately 0.01% to

approximately 30%, approximately 0.02% to approximately 29%, approximately 0.03% to approximately 28%, approximately 0.04% to approximately 27%, approximately 0.05% to approximately 26%, approximately 0.06% to approximately 25%, approximately 0.07% to approximately 24%, approximately 0.08% to approximately 23%, approximately 0.09% to approximately 22%, approximately 0.1% to approximately 21%, approximately 0.2% to approximately 20%, approximately 0.3% to approximately 19%, approximately 0.4% to approximately 18%, approximately 0.5% to approximately 17%, approximately 0.6% to approximately 16%, approximately 0.7% to approximately 15%, approximately 0.8% to approximately 14%, approximately 0.9% to approximately 12%, or approximately 1% to approximately 10% w/w, w/v or v/v.

[0155] In some embodiments, the concentration of the compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, provided in the pharmaceutical compositions ranges from approximately 0.001% to approximately 10%, approximately 0.01% to approximately 5%, approximately 0.02% to approximately 4.5%, approximately 0.03% to approximately 4%, approximately 0.04% to approximately 3.5%, approximately 0.05% to approximately 3%, approximately 0.06% to approximately 2.5%, approximately 0.07% to approximately 2%, approximately 0.08% to approximately 1.5%, approximately 0.09% to approximately 1%, or approximately 0.1% to approximately 0.9% w/w, w/v or v/v.

[0156] In some embodiments, the amount the compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, provided in the pharmaceutical compositions is equal to or less than 10 g, 9.5 g, 9.0 g, 8.5 g, 8.0 g, 7.5 g, 7.0 g, 6.5 g, 6.0 g, 5.5 g, 5.0 g, 4.5 g, 4.0 g, 3.5 g, 3.0 g, 2.5 g, 2.0 g, 1.5 g, 1.0 g, 0.95 g, 0.9 g, 0.85 g, 0.8 g, 0.75 g, 0.7 g, 0.65 g, 0.6 g, 0.55 g, 0.5 g, 0.45 g, 0.4 g, 0.35 g, 0.3 g, 0.25 g, 0.2 g, 0.15 g, 0.1 g, 0.09 g, 0.08 g, 0.07 g, 0.06 g, 0.05 g, 0.04 g, 0.03 g, 0.02 g, 0.01 g, 0.009 g, 0.008 g, 0.007 g, 0.006 g, 0.005 g, 0.004 g, 0.003 g, 0.002 g, 0.001 g, 0.0009 g, 0.0008 g, 0.0007 g, 0.0006 g, 0.0005 g, 0.0004 g, 0.0003 g, 0.0002 g, or 0.0001 g.

[0157] In some embodiments, the amount of the compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, provided in the pharmaceutical compositions of the present disclosure is more than 0.0001 g, 0.0002 g, 0.0003 g, 0.0004 g, 0.0005 g, 0.0006 g, 0.0007 g, 0.0008 g, 0.0009 g, 0.001 g, 0.0015 g, 0.002 g, 0.0025 g, 0.003 g, 0.0035 g, 0.004 g, 0.0045 g, 0.005 g, 0.0055 g, 0.006 g, 0.0065 g, 0.007 g, 0.0075 g, 0.008 g, 0.0085 g, 0.009 g, 0.0095 g, 0.01 g, 0.015 g, 0.02 g, 0.025 g, 0.03 g, 0.035 g, 0.04 g, 0.045 g, 0.05 g, 0.055 g, 0.06 g, 0.065 g, 0.07 g, 0.075 g, 0.08 g, 0.085 g, 0.09 g, 0.095 g, 0.1 g, , 0.15 g, 0.2 g, 0.25 g, 0.3 g, 0.35 g, 0.4 g, 0.45 g, 0.5 g, 0.55 g, 0.6 g, 0.65 g, 0.7 g, 0.75 g,

0.8 g, 0.85 g, 0.9 g, 0.95 g, 1 g, 1.5 g, 2 g, 2.5 g, 3 g, 3.5 g, 4 g, 4.5 g, 5 g, 5.5 g, 6 g, 6.5g, 7 g, 7.5g, 8 g, 8.5 g, 9 g, 9.5 g, or 10 g.

[0158] In some embodiments, the amount of the compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, provided in the pharmaceutical compositions ranges from 0.0001-10 g, 0.0005-9 g, 0.001-8 g, 0.005-7 g, 0.01-6 g, 0.05-5 g, 0.1-4 g, 0.5-4 g, or 1-3 g.

[0159] In some embodiments, the method includes administering compound A19 or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof by subcutaneous injection.

[0160] In some embodiments, the method includes administering the HGF/MET positive modulator by oral dosage form.

[0161] In some embodiments, the method includes administering Compound **2a** or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof by oral dosage form.

[0162] In some embodiments, the method includes administering Compound **1a** or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof by oral dosage form.

[0163] In some embodiments, the method includes administering Compound **5a** or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof by oral dosage form.

[0164] In some embodiments, the method includes administering Compound **6a** or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof by oral dosage form.

[0165] In some embodiments, the method includes administering Compound **7a** or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof by oral dosage form.

Kits/Articles of Manufacture

[0166] For use in the therapeutic applications described herein, kits and articles of manufacture are also provided. In some embodiments, such kits comprise a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers are formed from a variety of materials such as glass or plastic.

[0167] The articles of manufacture provided herein contain packaging materials. Packaging materials for use in packaging pharmaceutical products include those found in, *e.g.*, U.S. Pat. Nos. 5,323,907, 5,052,558 and 5,033,252. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, inhalers, pumps, bags, vials, containers, syringes, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment. For example, the container(s) includes one or

more compounds described herein, optionally in a composition or in combination with another agent as disclosed herein. The container(s) optionally have a sterile access port (for example the container is an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). Such kits optionally comprise a compound with an identifying description or label or instructions relating to its use in the methods described herein.

[0168] For example, a kit typically includes one or more additional containers, each with one or more of various materials (such as reagents, optionally in concentrated form, and/or devices) desirable from a commercial and user standpoint for use of a compound of Formula (I), or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. Non-limiting examples of such materials include, but not limited to, buffers, diluents, filters, needles, syringes, carriers, packages, containers, vials, and/or tube labels listing contents and/or instructions for use, and package inserts with instructions for use. A set of instructions will also typically be included. A label is optionally on or associated with the container. For example, a label is on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself, or a label is associated with a container when it is present within a receptacle or carrier that also holds the container, *e.g.*, as a package insert. In addition, a label is used to indicate that the contents are to be used for a specific therapeutic application. In addition, the label indicates directions for use of the contents, such as in the methods described herein. In certain embodiments, the pharmaceutical compositions are presented in a pack or dispenser device which contains one or more unit dosage forms containing a compound provided herein. The pack for example contains metal or plastic foil, such as a blister pack. Or, the pack or dispenser device is accompanied by instructions for administration. Or, the pack or dispenser is accompanied with a notice associated with the container in form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. Such notice, for example, is the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product insert. In some embodiments, compositions containing a compound of Formula (I), or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, formulated in a compatible pharmaceutical carrier are prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Methods of Use/Treatments

[0169] Embodiments of the present disclosure provide a method for modulating hepatocyte growth factor in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound as disclosed herein (*e.g.*, a compound of Formula (I) or

compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof). In some embodiments, a compound described herein activates hepatocyte growth factor. In some embodiments, a compound as described herein positively modulates hepatocyte growth factor activity. Modulation (*e.g.*, inhibition or activation) of hepatocyte growth factor can be assessed and demonstrated by a wide variety of ways known in the art. Kits and commercially available assays can be utilized for determining whether and to what degree hepatocyte growth factor has been modulated (*e.g.*, inhibited or activated).

[0170] In some embodiments, provided herein are compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt thereof, for use in modulating hepatocyte growth factor in a subject in need thereof. In some embodiments, provided herein are compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for modulating hepatocyte growth factor in a subject in need thereof.

[0171] Applicant has discovered that the compounds of Formula (I) or compound A19 show promising activity related to certain diseases of interest. Accordingly, in one aspect, provided herein is a method for modulating hepatocyte growth factor in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, provided herein is a method for activating hepatocyte growth factor in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, provided herein is a method for positively modulating hepatocyte growth factor activity in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof.

[0172] In certain more specific embodiments, the modulating comprises treating, or reducing the symptoms of, a disease, condition or injury. In some embodiments, the disease, condition, or injury is a neuroinflammatory condition. The neuroinflammatory condition may be multiple sclerosis, stroke, a frontotemporal dementia, an encephalopathy, or an encephalitis.

[0173] In some embodiments, the neuroinflammatory condition is a multiple sclerosis (MS). Multiple sclerosis is a chronic inflammatory disease of the central nervous system (CNS) that is characterized pathologically by demyelination, gliosis, neuro-axonal damage and inflammation. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer

thereof, reduces inflammation associated with MS. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces glial cell death associated with demyelination. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces gliosis. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces neuroaxonal damage. In some embodiments, administering to a subject having multiple sclerosis an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, results in longitudinal improvement in the Expanded Disability Status Scale (EDSS), improvement in the MS Functional Composite (MSFC) score, improvement in the volumetric MRI of neurological structures (thalamus, upper cervical cord) or of lesions, or plasma biomarkers of neuro- or myelodegeneration, and/or a reduction in disease incidence flare-ups.

[0174] In some embodiments, the neuroinflammatory condition is stroke. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces inflammation associated with the stroke. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces inflammation caused by the stroke. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces the risk of a subject experiencing a stroke. In some embodiments, administering to a subject suffering from stroke an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, results in an improvement in volumetric MRI of affected structures, and/or an improvement in psychometric scales measuring including language and/or motor function.

[0175] In some embodiments, the neuroinflammatory condition is a frontotemporal dementia. In some embodiments, the neuroinflammatory condition is an idiopathic frontotemporal dementia. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces inflammation associated with the frontotemporal dementia. In some embodiments, administering to a subject an effective amount of a compound

of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces inflammation caused by the frontotemporal dementia. In some embodiments, administering to a subject suffering from frontotemporal dementia an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, results in an improvement in TAR DNA binding protein 43 (TDP-43) levels, serum progranulin (PRGN) and NfL levels, longitudinal volumetric MRI, and/or psychometric scales.

[0176] In some embodiments, the neuroinflammatory condition is, is associated with, or is caused by, an encephalopathy. In some embodiments, the neuroinflammatory condition is, is associated with, or is caused by, an encephalopathy, such as small vessel encephalopathy ("Binswanger's disease"), multi-infarct dementia, or stroke. In some embodiments, the neuroinflammatory condition is, is associated with, or is caused by, an encephalopathy such as chronic traumatic encephalopathy. In some embodiments, the neuroinflammatory condition is, is associated with, or is caused by, an encephalopathy resulting from liver organ dysfunction leading to accumulation of neurotoxins (a hepatic encephalopathy) and/or kidney organ dysfunction leading to accumulation of neurotoxins (a uremic encephalopathy). In some embodiments, the neuroinflammatory condition is, is associated with, or is caused by, an encephalopathy resulting from peripheral sepsis. In some embodiments, the neuroinflammatory condition is, is associated with, or is caused by, an encephalopathy resulting from a metabolic disorder (a metabolic encephalopathy) including but not limited to diabetes. In some embodiments, the neuroinflammatory condition is, is associated with, or is caused by, an encephalopathy, such as a hypoxic encephalopathy. In some embodiments, the neuroinflammatory condition is, is associated with, or is caused by, Hashimoto encephalopathy, a rare disorder characterized by impaired brain function.

[0177] In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces inflammation associated with the encephalopathy. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces inflammation associated with or caused by the encephalopathy. In some embodiments, administering to a subject suffering from an encephalopathy an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, results in an improvement in psychometric scales of brain function and/or measures of disease leading to the brain dysfunction.

[0178] In some embodiments, the neuroinflammatory condition is an encephalitis. In some embodiments, the neuroinflammatory condition is an encephalitis, such as an autoimmune encephalitis, a viral encephalitis or a bacterial encephalitis. In some embodiments, the autoimmune encephalitis is an NMDAR encephalitis. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces inflammation associated with the encephalitis. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces inflammation caused by the encephalitis. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, reduces hypoxia associated with the inflammation of the encephalitis. In some embodiments, administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, improves neuronal survival. In some embodiments, administering to a subject suffering from an encephalitis an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, results in a reduction in intracranial pressure, CSF expression of inflammatory cytokines: IL-1, CXCL10, CCL3, IL-10, CCL22, and IL-6, and/or an improvement in psychometric scales of cognition and memory.

[0179] Also provided herein is a method for reducing inflammation associated with the neuroinflammatory condition comprising administering to a subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In various embodiments, the neuroinflammatory condition is associated hypoxia caused by inflammation.

[0180] In some embodiments, the neuroinflammation is not caused by peripheral inflammation or a disease or disorder of the peripheral nervous system. In some embodiments, the neuroinflammation is not cause by a dementia. In some embodiments, the neuroinflammation is not caused by a neurodegenerative disease. In some embodiments, the neuroinflammation is not caused by Alzheimer's disease, mild cognitive impairment, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, spinal cord injury, traumatic brain injury, or sensorineural hearing and vision loss. In some embodiments, the subject being treated for neuroinflammation is not suffering from a dementia. In some embodiments, the subject being treated for neuroinflammation is not suffering from a neurodegenerative disease. In some embodiments, the subject being treated for neuroinflammation is not suffering from Alzheimer's disease, mild cognitive impairment, Parkinson's disease, Huntington's disease, amyotrophic

lateral sclerosis, spinal cord injury, traumatic brain injury, and/or sensorineural hearing and vision loss.

[0181] In some embodiments, the neuroinflammation is not associated with peripheral inflammation or a disease or disorder of the peripheral nervous system. In some embodiments, the neuroinflammation is not associated with a dementia. In some embodiments, the neuroinflammation is not associated with a neurodegenerative disease. In some embodiments, the neuroinflammation is not associated with Alzheimer's disease, mild cognitive impairment, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, spinal cord injury, traumatic brain injury, or sensorineural hearing and vision loss. In some embodiments, the subject being treated for neuroinflammation is not suffering from a dementia.

[0182] In some embodiments, the disclosure provides methods of modulating protein activity (*e.g.*, hepatocyte growth factor activity) in a subject including but not limited to rodents and mammal (*e.g.*, human) by administering into the subject an effective amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, modulation of hepatocyte growth factor is activation of hepatocyte growth factor. In some embodiments, the percentage modulation exceeds 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%. In some embodiments, the percentage of inhibiting exceeds 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%.

[0183] In some embodiments, the disclosure provides methods of modulating hepatocyte growth factor activity in a cell by contacting said cell with an amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, sufficient to modulate the activity of hepatocyte growth factor. In some embodiments, the disclosure provides methods of modulating hepatocyte growth factor activity in a tissue by contacting said tissue with an amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, sufficient to modulate the activity of hepatocyte growth factor in the tissue. In some embodiments, the disclosure provides methods of modulating hepatocyte growth factor activity in an organism by contacting said organism with an amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, sufficient to modulate the activity of hepatocyte growth factor in the organism. In some embodiments, the disclosure provides methods of modulating hepatocyte growth factor activity in an animal by contacting the animal with an amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, sufficient to modulate the activity of hepatocyte growth factor in the animal. In some embodiments, the disclosure provides methods of modulating hepatocyte growth factor activity in a mammal by contacting the mammal with an

amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, sufficient to modulate the activity of hepatocyte growth factor in the mammal. In some embodiments, the disclosure provides methods of modulating hepatocyte growth factor activity in a human by contacting the human with an amount of a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, sufficient to modulate the activity of hepatocyte growth factor in the human. In other embodiments, the present disclosure provides methods of treating a disease mediated by hepatocyte growth factor activity in a subject in need of such treatment. In some variations, modulation of hepatocyte growth factor by a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, involves activation of hepatocyte growth factor.

[0184] Other embodiments provide methods for combination therapies in which a therapeutic agent known to modulate other pathways, or other components of the same pathway, or even overlapping sets of target enzymes are used in combination with a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In one aspect, such therapy includes but is not limited to the combination of one or more compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, with therapeutic agents, therapeutic antibodies, and other forms of treatment, to provide a synergistic or additive therapeutic effect.

[0185] Many therapeutic agents are presently known in the art and can be used in combination with the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, a subject having multiple sclerosis also be treated with a therapeutic agent such as a beta interferon, glatiramer, cladribine, dimethyl fumarate, diroximel fumarate, fingolimod, monomethyl fumarate, ozanimod, Siponimod, teriflunomide, alemtuzumab, mitoxantrone, natalizumab, ocrelizumab or a steriod.

[0186] In some embodiments, a compound provided herein is administered to patients having or recovering from a stroke and who are also being treated with a therapeutic agent such as a tissue plasminogen activator, or a calcium antagonist for the prevention and therapy of secondary arteriole spams.

[0187] In some embodiments, a compound provided herein is administered in combination with a systemic or topical corticosteroid, abatacept, tocilizumab, for example, in a subject having a frontotemporal dementia is also being treated with a therapeutic agent such as an antidepressant or an anti-psychotic. In some embodiments, the therapeutic agent is selected from antidepressants/anxiolytics and/or antipsychotic medicines. Antidepressants may include,

but are not limited to, selective serotonin reuptake inhibitors (SSRIs) (e.g., duloxetine, venlafaxine, citalopram, paroxetine or escitalopram). Some embodiments of the methods described herein may include use or administration of antipsychotic medicines such as aripiprazole (Abilify), haloperidol (Haldol), olanzapine (Zyprexa), and risperidone (Risperdal).

[0188] In some embodiments, a subject having an encephalopathy is also being treated with a therapeutic agent such as an anti-inflammatory agent, such as acetaminophen, ibuprofen or naproxen sodium, an anti-viral agent such as acyclovir, ganciclovir or foscarnet, or an antibiotic.

[0189] In some embodiments, a subject having an encephalitis is also being treated with a therapeutic agent, such as an anti-inflammatory agent, such as acetaminophen, ibuprofen or naproxen sodium, a steroid(s), an anti-viral agent, or intravenous antibodies (e.g., IVIg) or plasma exchange.

[0190] Many therapeutic agents are presently known in the art and can be used in combination with the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof. In some embodiments, the therapeutic agent is selected from a gabapentinoid (e.g., pregabalin or gabapentin), duloxetine, antidepressants (e.g., amitriptyline, desipramine, orimipramine), an opioid-like medication (e.g, tramadol or tapentadol), , desvenlafaxine, a topical agent (e.g., lidocaine patches and capsaicin cream), an opioid (e.g., oxycodone or morphine) or selective serotonin-norepinephrine reuptake inhibitors (e.g., duloxetine, venlafaxine, citalopram, paroxetine or escitalopram). In some embodiments, the therapeutic agent is selected from pregabalin, gabapentin), duloxetine, amitriptyline, tramadol, tapentadol, oxycodone, morphine, citalopram, paroxetine or escitalopram).

[0191] In some embodiments, the therapeutic agent is selected from antidepressants and/or antipsychotic medicines. Antidepressants may include, but are not limited to, selective serotonin reuptake inhibitors (SSRIs). Some embodiments of the methods described herein may include use or administration of antipsychotic medicines such as aripiprazole (Abilify), haloperidol (Haldol), olanzapine (Zyprexa), and risperidone (Risperdal).

[0192] In some embodiments, the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are formulated or administered in conjunction with liquid or solid tissue barriers also known as lubricants. Examples of tissue barriers include, but are not limited to, polysaccharides, polyglycans, seprafilm, interceed and hyaluronic acid.

[0193] Further therapeutic agents that can be combined with a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are found in Goodman and Gilman's "The Pharmacological Basis of Therapeutics" Tenth Edition

edited by Hardman, Limbird and Gilman or the Physician's Desk Reference, both of which are incorporated herein by reference in their entirety.

[0194] The compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, can be used in combination with the therapeutic agents disclosed herein depending on the condition being treated. Hence, in some embodiments the one or more compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, will be co-administered with other therapeutic agents as described above. When used in combination therapy, the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are administered with the second therapeutic agent simultaneously or separately. This administration in combination can include simultaneous administration in the same dosage form, simultaneous administration in separate dosage forms, and separate administration. That is, a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, and any of the therapeutic agents described above can be formulated together in the same dosage form and administered simultaneously. Alternatively, a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, and any of the therapeutic agents described above can be simultaneously administered, wherein both are present in separate formulations. In another alternative, a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, can be administered just followed by and any of the therapeutic agents described above, or vice versa. In some embodiments of the separate administration protocol, a compound of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, and any of the therapeutic agents described above are administered a few minutes apart, or a few hours apart, or a few days apart.

[0195] The examples and preparations provided below further illustrate and exemplify the compounds of Formula (I) or compound A19, or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, and methods of preparing such compounds. It is to be understood that the scope of the present disclosure is not limited in any way by the scope of the following examples and preparations. In the following examples, and throughout the specification and claims, molecules with a single stereocenter, unless otherwise noted, exist as a racemic mixture. Those molecules with two or more stereocenters, unless otherwise noted, exist as a racemic mixture of diastereomers. Single enantiomers/diastereomers may be obtained by methods known to those skilled in the art.

EXAMPLES

[0196] The following examples are provided for exemplary purposes. Methods for preparation of compounds of Formula (I), or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, are provided herein or can be derived by one of ordinary skill in the art.

[0197] The examples and preparations provided below further illustrate and exemplify the compounds of the present disclosure and methods for testing such compounds. It is to be understood that the scope of the present disclosure is not limited in any way by the scope of the following examples.

[0198] The chemical reactions in the Examples described can be readily adapted to prepare a number of other compounds disclosed herein, and alternative methods for preparing the compounds of this disclosure are deemed to be within the scope of this disclosure. For example, the synthesis of non-exemplified compounds according to the present disclosure can be performed by modifications apparent to those skilled in the art, for example by appropriately protecting interfering groups, by utilizing other suitable reagents known in the art other than those described, or by making routine modification of reaction conditions, reagents, and starting materials. Alternatively, other reactions disclosed herein or known in the art will be recognized as having applicability for preparing other compounds of the present disclosure.

[0199] Unless indicated otherwise in the following Examples, the compounds are isolated as a racemic mixture.

[0200] The following abbreviations may be relevant for the application.

Abbreviations

AcOH: acetic acid

CAN: ceric ammonium nitrate

DAST: diethylaminosulfur trifluoride

DCM: dichloromethane

DIPEA: N,N-diisopropylethylamine

DMEM: Dulbecco's Modified Eagle Medium

DMF: dimethylformamide

DMSO: dimethylsulfoxide

EMEM: Eagle's Minimum Essential Medium

EtOAc: ethyl acetate

EtOH: ethanol

FBS: fetal bovine serum

Fmoc: fluorenylmethoxycarbonyl

HATU: (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate

LC/MS: liquid chromatography-mass spectrometry

Me: methyl

MeOH: methanol

PBS: phosphate buffered saline

Pic-BH_3: picoline borane

PMB: para-methoxybenzyl ether

Prep HPLC: preparative high performance liquid chromatography

rt or RT: room temperature

TFA: trifluoroacetic acid

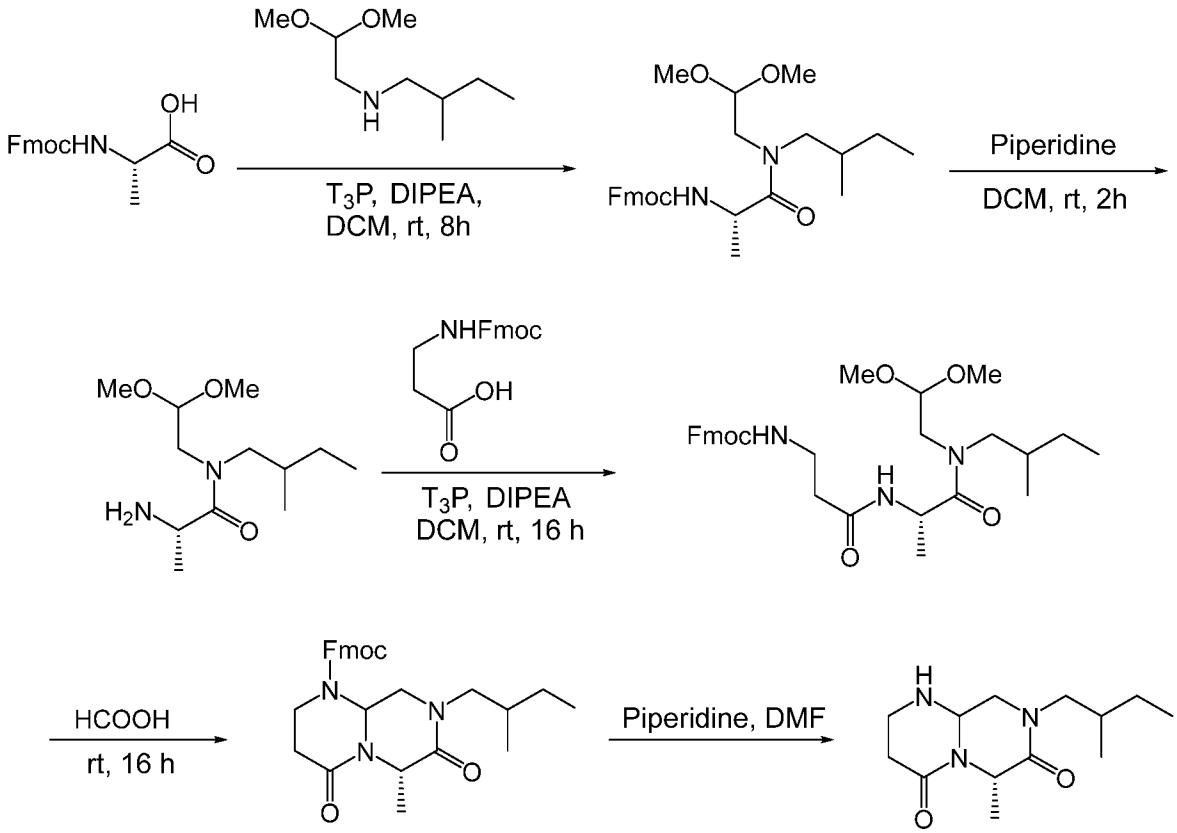
TLC: thin layer chromatography

T_3P: Propanephosphonic acid anhydride

Synthetic Examples

Example S1. Synthesis of (6S)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione. The synthetic route for preparing this starting material compound is shown in Scheme 1.

Scheme 1.



[0201] Step 1: Synthesis of (9H-fluoren-9-yl)methyl (2S)-1-((2,2-dimethoxyethyl)(2-methylbutyl)amino)-1-oxopropan-2-ylcarbamate. To a stirred solution of compound (S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)propanoic acid (5.0 g, 16.07) in dichloromethane (100 mL) was added T_3P (15.2 mL, 24.1) and DIPEA (5.6 mL, 32.1 mmol) at room temperature.

The reaction mixture was stirred at room temperature for 15 min and N-(2,2-dimethoxyethyl)-2-methylbutan-1-amine (2.81 g, 32.1 mmol.) was added, and stirring was continued at room temperature for 8 hours. The reaction was monitored by TLC. After completion, the reaction mixture was quenched with ice cold water (100 mL) and extracted with dichloromethane (2 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give crude compound. The crude compound was purified by flash column chromatography (100-200 mesh silica gel, eluted with 40% ethyl acetate in petroleum ether) to afford pure compound (9H-fluoren-9-yl)methyl (2S)-1-((2,2-dimethoxyethyl)(2-methylbutyl)amino)-1-oxopropan-2-ylcarbamate (5.2 g, 69.1%) as a gummy compound.

[0202] Step 2: Synthesis of (2S)-2-amino-N-(2,2-dimethoxyethyl)-N-(2-methylbutyl)propenamide. To a stirred solution of (9H-fluoren-9-yl)methyl (2S)-1-((2,2-dimethoxyethyl)(2-methylbutyl)amino)-1-oxopropan-2-ylcarbamate (34.0 g, 72.6 mmol) in DMF (230 mL) was added 20% piperidine in DMF (70 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 hours. The reaction was monitored by TLC. After completion of the reaction, excess DMF (100 mL) was added, then washed with excess n-hexane (3 × 200 mL). The DMF layer was collected and poured in ice cold water (1000 mL), then extracted with 10% methanol-dichloromethane (3 × 500 mL). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give (2S)-2-amino-N-(2,2-dimethoxyethyl)-N-(2-methylbutyl)propanamide (20.4 g, 68.4%) as a gummy solid.

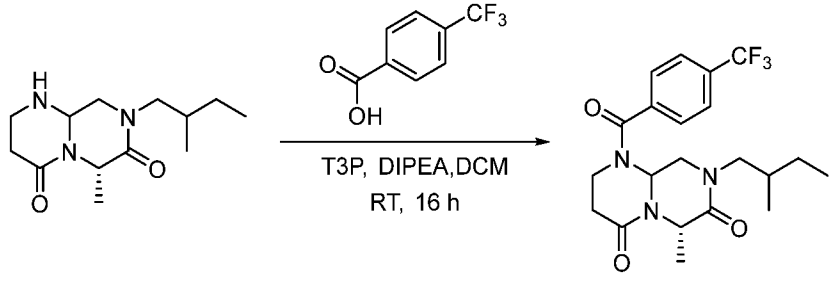
[0203] Step 3: Synthesis of (9H-fluoren-9-yl)methyl3-((2S)-1-((2,2-dimethoxyethyl)(2-methylbutyl)amino)-1-oxopropan-2-ylamino)-3-oxopropylcarbamate. To a stirred solution of 3-(((9H-fluoren-9-yl) methoxy)carbonylamino)propanoic acid (20.2 g, 81.2 mmol) stirred in dichloromethane at room temperature (500 mL) was added T_3P (80 mL, 121.8 mmol) and DIPEA (28.6 mL, 160.4 mmol), and the mixture was stirred for 10 minutes. To this (2S)-2-amino-N-(2,2-dimethoxyethyl)-N-(2-methylbutyl)propanamide (25.53 81.2 mmol) was added and stirring was continued at room temperature for 16 hours. The reaction progress was monitored by TLC. After completion, the reaction mixture was quenched with water (500 mL) and the mixture was extracted with dichloromethane (2 × 500 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude product. The crude compound was purified by flash column chromatography (100-200 mesh Silica gel, eluted with 70% ethyl acetate in petroleum ether) to afford pure compound (9H-fluoren-9-yl)methyl3-((2S)-1-((2,2-dimethoxyethyl)(2-methylbutyl)amino)-1-oxopropan-2-ylamino)-3-oxopropylcarbamate (21.2 g, 78.6%) as a gummy compound.

[0204] Step 4: Synthesis of (6S)-(9H-fluoren-9-yl)methyl 6-methyl-8-(2-methylbutyl)-4,7-dioxooctahydro-1H-pyrazino[1,2-a]pyrimidine-1-carboxylate. To a stirred solution of (9H-fluoren-9-yl)methyl 3-((2S)-1-((2,2-dimethoxyethyl)(2-methylbutyl)amino)-1-oxopropan-2-ylamino)-3-oxopropylcarbamate (21.0 g, 38.9 mmol) was added formic acid (105 mL). The reaction mixture was stirred at room temperature for 12 hours. The reaction progress was monitored by TLC. After completion, the reaction mixture was concentrated under reduced pressure to give crude compound. The crude compound was taken up in saturated aqueous $NaHCO_3$ (200 mL) solution, then extracted with ethyl acetate (3 × 500 mL). The combined organic layers were washed with brine solution (500 mL), then the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was purified by flash column chromatography (100-200 mesh silica gel, eluted with 50% ethyl acetate in petroleum ether) to afford pure compound (6S)-(9H-fluoren-9-yl)methyl 6-methyl-8-(2-methylbutyl)-4,7-dioxooctahydro-1H-pyrazino[1,2-a]pyrimidine-1-carboxylate (25 g, 69.0%) as a gum.

[0205] Step 5: (6S)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione. To a stirred solution of (6S)-(9H-fluoren-9-yl)methyl 6-methyl-8-(2-methylbutyl)-4,7-dioxooctahydro-1H-pyrazino[1,2-a]pyrimidine-1-carboxylate (14.0 g, 29.4 mmol) at 0°C in DMF (70 mL) was added 20% piperidine in DMF (30 mL). The reaction mixture was allowed to warm to room temperature and stirred for 2 hours. The reaction was monitored by TLC. After complete consumption of starting material, additional DMF was added (50 mL), then the mixture was washed with excess n-hexane (3 × 200 mL). The DMF layer was poured into ice cold water (1000 mL) and extracted with 10% methanol-dichloromethane (3 × 500 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to provide the desired crude compound (6S)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (6.25 g, 83.8%) as a solid.

Example S2. Synthesis of Compound 1a. The synthetic route for preparing Compound **1a** is shown in Scheme 2.

Scheme 2.

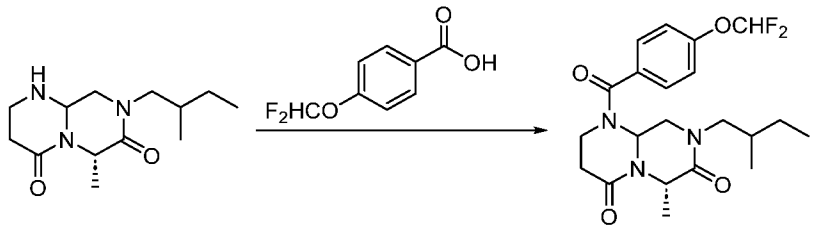


1a

[0206] To a solution of 4-(trifluoromethyl)benzoic acid (0.232 g, 0.91 mmol) stirred in dichloromethane (20 mL) at room temperature was added T_3P (1.2 mL, 1.37 mmol) and DIPEA (0.42 mL, 1.82 mmol), and the mixture was stirred for 15 minutes. To this (6S)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (0.310 g, 0.91 mmol) was added and stirring was continued for 8 hours. The reaction progress was monitored by TLC. After reaction completion, the mixture was quenched with water (50 mL) and extracted with dichloromethane (2 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was purified by Prep HPLC. The pure fractions were combined and concentrated under reduced pressure, then lyophilized to afford Compound **1a** (0.340 g, 65.3%) as a solid. Prep HPLC method: Mobile phase A: 10 mM ammonium bicarbonate in water; Mobile phase B: acetonitrile; Column: X-Select phenyl hexyl (150 × 19mm 5µ); Flow: 16 mL/min. MS (ESI) m/z $[M+H]^+$: 426.05.

Example S3. Synthesis of Compound 2a. The synthetic route for preparing Compound **2a** is shown in Scheme 3.

Scheme 3.

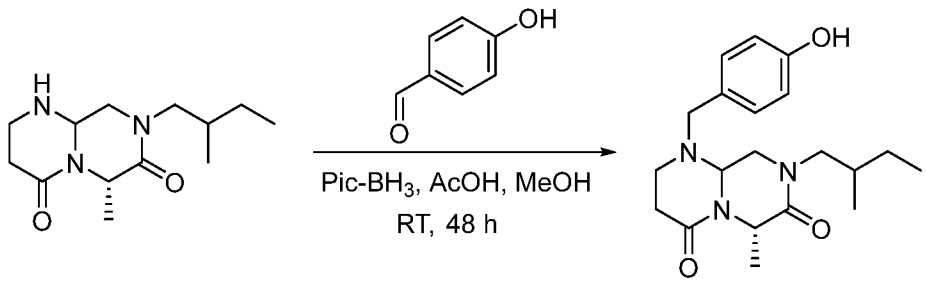


2a

[0207] To a solution of 4-(difluoromethoxy) benzoic acid (0.37 g, 1.968 mmol) in dichloromethane (15 mL) at room temperature was added DIPEA (0.8 ml, 5.904 mmol) and T_3P (2.0 mL, 3.936 mmol). The mixture was stirred for 30 min, then (6S)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (0.4 g, 1.578 mmol) was added, and stirring was continued for 16 hours. Progress of the reaction was monitored by TLC and LC/MS. The reaction mixture was diluted with dichloromethane (100 mL) and washed with water (50 mL) and saturated sodium chloride solution (50 mL), then dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by Prep HPLC. The pure fractions were collected and lyophilized to afford Compound **2a** (380 mg 46%) as a solid. Prep HPLC condition: Mobile phase A: 10 mM ammonium bicarbonate in water; Mobile phase B: Acetonitrile; Column: Kromosil phenyl (150 × 25 mm 10µ); Flow: 25 mL/min. MS (ESI) m/z $[M+H]^+$: 424.11.

Example S4. Synthesis of Compound 3a. The synthetic route for preparing Compound **3a** is shown in Scheme 4.

Scheme 4.

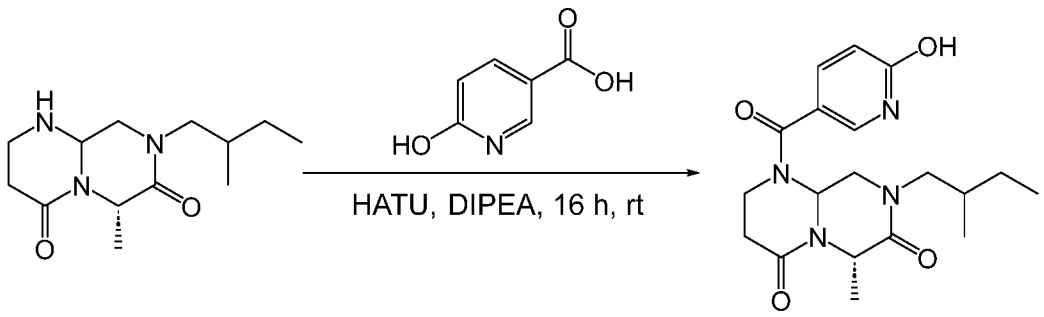


3a

[0208] To a solution of (6S)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (0.500 g, 1.97 mmol) stirred in methanol (20 mL) at room temperature was added 4-hydroxybenzaldehyde (0.289 g, 1.97 mmol) and acetic acid (0.23 mL, 3.95 mmol). The reaction mixture was stirred at room temperature for 5 minutes. To this picoline borane (0.253 g, 2.37 mmol) was added, and stirring was continued for 48 hr. The reaction progress was monitored by TLC. After completion, the reaction mixture was quenched with ice cold water (50 mL), and the mixture was extracted with 10% methanol-dichloromethane (3 × 40 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was purified by Prep HPLC. The pure fractions were combined and concentrated under reduced pressure, then lyophilized to afford Compound **3a** (0.180 g, 46.09%) as a solid. Prep HPLC Method: Mobile Phase A: 10 mM ammonium bicarbonate in water; Mobile Phase B: Acetonitrile; Column: Kromosil Phenyl (150 × 25 mm 10μ); Flow: 25 mL/min. MS (ESI) m/z $[M+H]^+$: 360.11.

Example S5. Synthesis of Compound 4a. The synthetic route for preparing Compound **4a** is shown in Scheme 5.

Scheme 5.



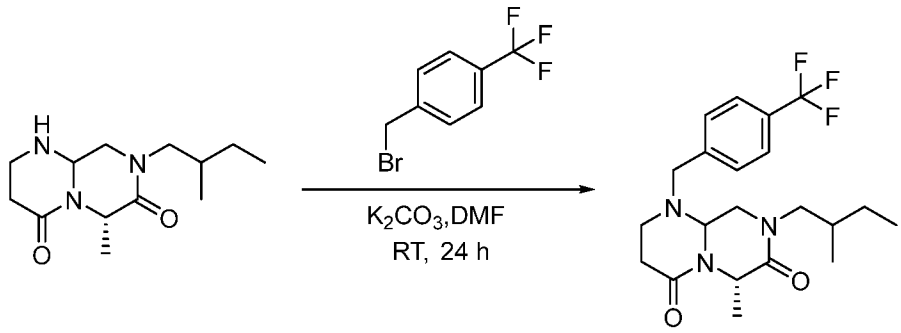
4a

[0209] To a solution of 6-hydroxynicotinic acid (0.340 g 2.446 mmol) in DMF (15 mL) at room temperature was added DIPEA (1.30 mL, 7.338 mmol) and HATU (1.39 g, 3.669 mmol). The resulting reaction mixture was stirred for 30 min, then (6S)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (0.495 g, 1.956 mmol.)

was added, and the mixture was stirred for 16 hours. Progress of the reaction was monitored by TLC and LC/MS (TLC system: 10% methanol/dichloromethane, R*f*: 0.15, Detection: UV). The reaction mixture was quenched with cold water (100 mL) and extracted with 10% methanol/dichloromethane (3 × 100 mL).The combined organic layers were washed with cold water (50 mL) and cold brine solution (50 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by Prep HPLC. The pure fractions were collected and lyophilized to afford Compound **4a** (160 mg, 21.8%) as a solid. Prep HPLC Method: Mobile Phase A: 0.01 mM ammonium bicarbonate in water; Mobile Phase B: acetonitrile; Column: X-Select phenyl hexyl (150 × 19mm, 5µ); Flow: 15 mL/min. MS (ESI) m/z $[M+H]^+$: 375.05.

Example S6. Synthesis of Compound 5a. The synthetic route for preparing Compound **5a** is shown in Scheme 6.

Scheme 6.

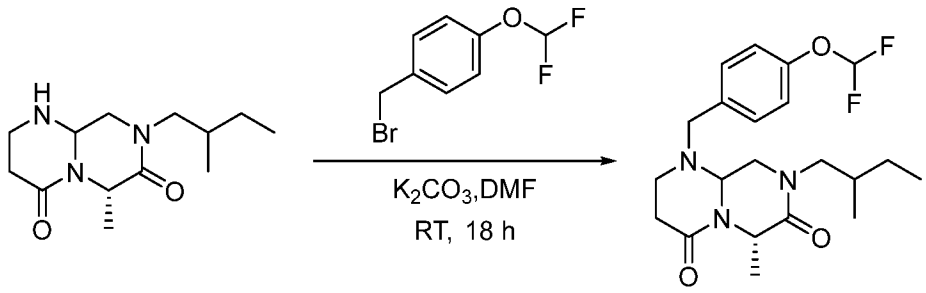


5a

[0210] To a solution of (6S)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (0.5 g, 1.97 mmol) and 1-(bromomethyl)-4-(trifluoromethyl)benzene (0.470 g, 1.97 mmol) stirred in DMF (20 mL) at room temperature was added K_2CO_3 (0.546 g, 3.95 mmol), and the mixture was stirred for 8 hr. The reaction progress was monitored by TLC. After completion, the mixture was quenched with water (100 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was purified by Prep HPLC. The pure fractions were combined and concentrated under reduced pressure, then lyophilized to afford Compound **5a** (0.270 g, 63.8%) as a gum. Prep HPLC Method: Mobile Phase A: 10 mM ammonium bicarbonate in water; Mobile Phase B: Acetonitrile; Column: Kromosil C_{18} (150 × 25mm 10µ); Flow: 25 mL/min. MS (ESI) m/z $[M+H]^+$: 412.2.

Example S7. Synthesis of Compound 6a. The synthetic route for preparing Compound **6a** is shown in Scheme 7.

Scheme 7.

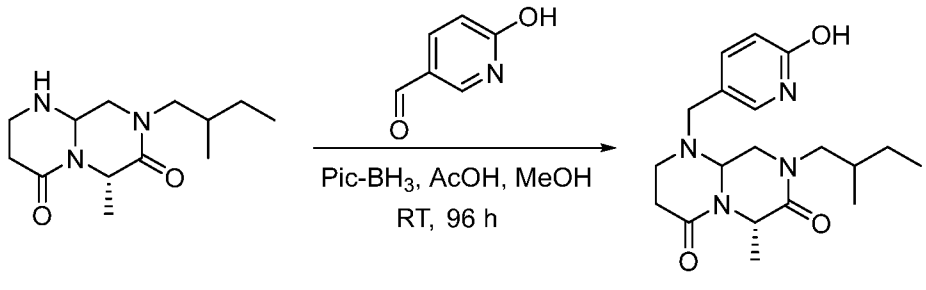


6a

[0211] To a solution of (6S)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (0.500 g, 1.97 mmol) and 1-(bromomethyl)-4-(difluoromethoxy)benzene (0.466 g, 1.97 mmol) stirred in DMF (20 mL) at room temperature was added K_2CO_3 (0.546 g, 9.95 mmol). The reaction mixture was stirred at room temperature for 18 hr. The reaction progress was monitored by TLC. After completion, the reaction mixture was quenched with water (100 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was purified by Prep HPLC. The pure fractions were combined and concentrated under reduced pressure, then lyophilized to afford Compound **6a** (0.178 g, 41.5%) as a semi-solid. Prep HPLC Method: Mobile Phase A: 10 mM ammonium bicarbonate in water; Mobile Phase B: acetonitrile; Column: X-Select C_{18} (250 × 19mm, 5µ); Flow: 18 mL/min. MS (ESI) m/z $[M+H]^+$: 410.11.

Example S8. Synthesis of Compound 7a. The synthetic route for preparing Compound **7a** is shown in Scheme 8.

Scheme 8.



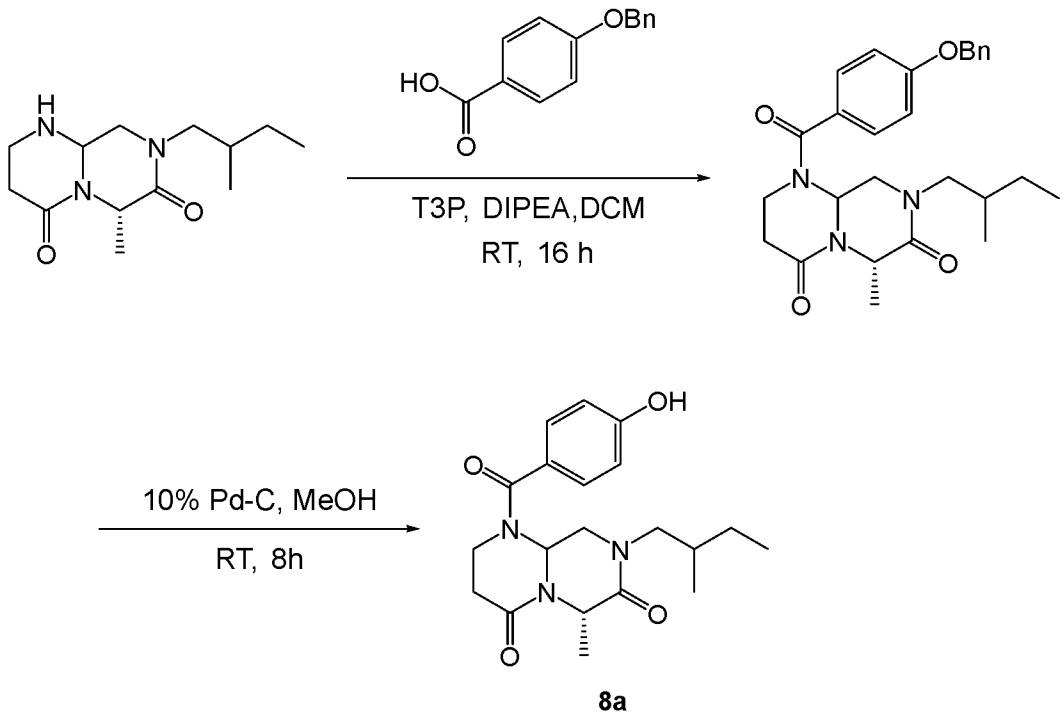
7a

[0212] To a solution of compound (6S)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (0.500 g, 1.97 mmol) stirred in methanol (20 mL) at room temperature was added 6-hydroxynicotinaldehyde (0.243 g, 1.97 mmol) and acetic acid (0.25 mL, 3.95 mmol), and the mixture was stirred for 5 min. To this picoline borane (0.318 g, 2.96 mmol) was added and stirring was continued for 96 hours. The reaction progress was monitored by TLC. After completion, the reaction mixture was quenched with ice cold water (50 mL) and extracted with 10% methanol-dichloromethane (3 × 40 mL). The combined organic

layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was purified by Prep HPLC. The pure fractions were collected and concentrated under reduced pressure, then lyophilized to afford Compound **7a** (0.164 g, 42%) as a solid. Prep HPLC Method: Mobile Phase A: 10 mM ammonium bicarbonate in water; Mobile Phase B: acetonitrile; Column: X-BRIDGE C_{18} (250 × 19 mm, 5μ); Flow: 18 mL/min. MS (ESI) m/z $[M+H]^+$: 361.11.

Example S9. Synthesis of Compound 8a. The synthetic route for preparing Compound **8a** is shown in Scheme 9.

Scheme 9.



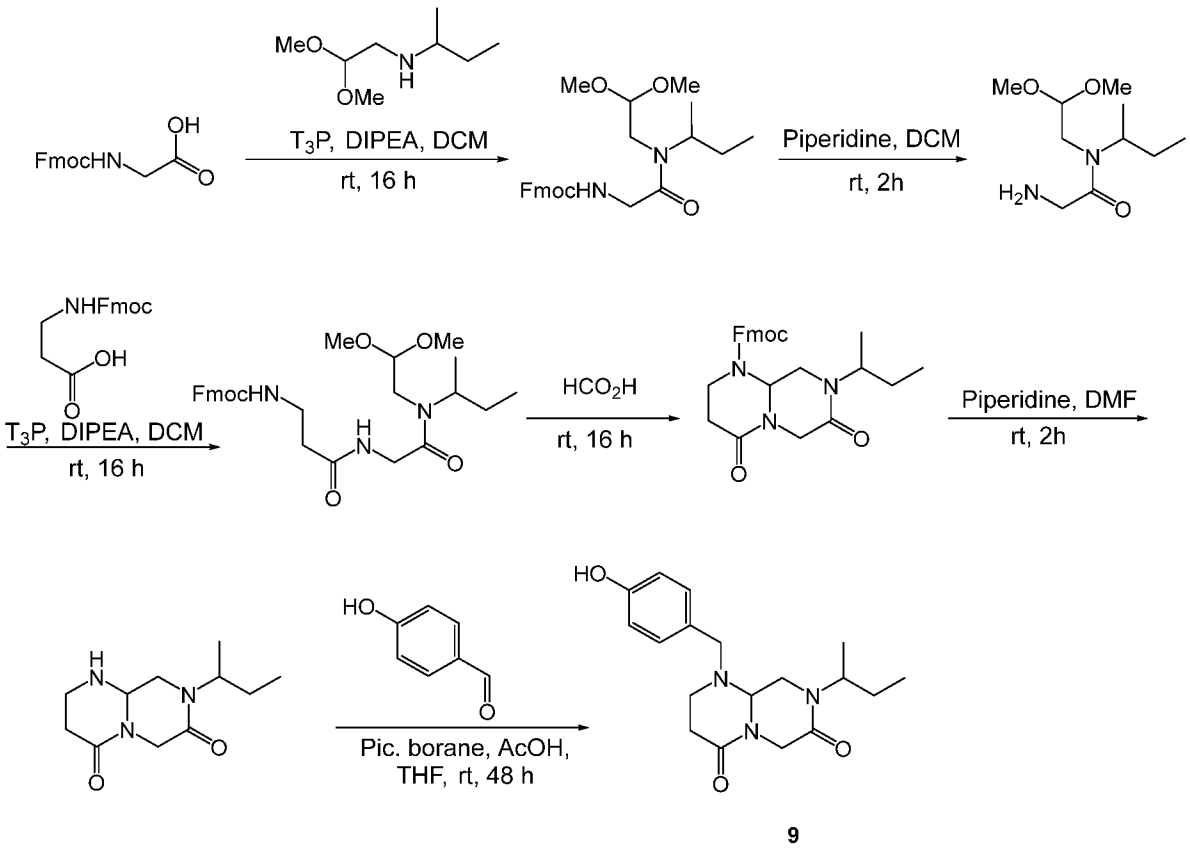
8a

[0213] Step 1: Synthesis of (6S)-1-(4-(benzyloxy)benzoyl)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione. To a solution of 4-(benzyloxy)benzoic acid (0.360 g, 1.42 mmol) stirred in dichloromethane (20 mL) at room temperature was added T_3P (1.2 mL, 1.7 mmol) and DIPEA (0.55 mL, 2.84 mmol), and the mixture was stirred for 15 min. To this (6S)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (0.400 g, 1.42 mmol) was added, and stirring was continued at room temperature for 16 hours. The reaction progress was monitored by TLC. After completion, the reaction mixture was quenched with water (50 mL) and extracted with dichloromethane (2 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give 0.9 g of crude material. Analysis of the crude material by LC/MS showed 54.59% of the desired product. The crude material was used in the next step without purification.

[0214] Step 2: Synthesis of Compound 8a. To a solution of (6S)-1-(4-(benzyloxy)benzoyl)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (0.900 g) stirred in methanol (20 mL) at room temperature was added 10% Pd-C (0.200 g), under N_2 atmosphere. The reaction mixture was stirred at room temperature under an H_2 balloon for 8 hr. The reaction progress was monitored by TLC. After completion, the reaction mixture was filtered through Celite and evaporated under reduced pressure to afford the crude compound. The crude compound was dissolved in dichloromethane (50 mL), washed with aqueous $NaHCO_3$ solution (20 mL) and brine solution (20 mL). The filtrate was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was triturated with diethyl ether to afford Compound **8a** (0.330 g, 82%) as a solid. MS (ESI) m/z $[M+H]^+$: 374.11.

Example S10. Synthesis of Compound 9. The synthetic route for preparing Compound **9** is shown in Scheme 10.

Scheme 10.



[0215] Step 1: Synthesis of (9H-fluoren-9-yl)methyl 2-(sec-butyl(2,2-dimethoxyethyl)amino)-2-oxoethylcarbamate. To a stirred solution of 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)acetic acid (10 g, 33.6 mmol) in dichloromethane (100 mL), cooled to 0 °C were added DIPEA (11.88 mL, 67.3 mmol), N-(2,2-dimethoxyethyl)butan-2-amine (10.84 g, 67.3 mmol) and T_3P (53.0 mL, 84.1 mmol), and the reaction mixture was stirred for 16

hours at room temperature. Reaction progress was monitored by TLC. After completion of the reaction, ice cold water (100 mL) was added and extracted with ethyl acetate (2 × 150 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain the desired crude product. The crude compound was purified by flash column chromatography (100-200 mesh silica gel) and eluted with 20-25% ethyl acetate in petroleum ether to afford (9H-fluoren-9-yl)methyl 2-(sec-butyl(2,2-dimethoxyethyl)amino)-2-oxoethylcarbamate (10.8 g, 72.9%) as a solid.

[0216] Step 2: Synthesis of 2-amino-N-sec-butyl-N-(2,2-dimethoxyethyl)acetamide. To a solution of (9H-fluoren-9-yl)methyl 2-(sec-butyl(2,2-dimethoxyethyl)amino)-2-oxoethylcarbamate (10.8 g, 24.5 mmol) in DMF (20 mL), cooled to 0 °C, was added piperidine (2.4 mL) and the reaction mixture was stirred at room temperature for 2 hours. Progress of the reaction was monitored by TLC. After TLC indicated complete consumption of starting material, the reaction mixture was diluted with petroleum ether (2 × 100 mL), then water was added and the mixture was separated. The aqueous layer was extracted with dichloromethane (2 × 150 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain the desired pure product 2-amino-N-sec-butyl-N-(2,2-dimethoxyethyl)acetamide (3.6 g, 67.2%) as a solid.

[0217] Step 3: Synthesis of (9H-fluoren-9-yl)methyl-3-(2-(sec-butyl(2,2-dimethoxyethyl)amino)-2-oxoethylamino)-3-oxopropylcarbamate. To a stirred solution of 2-amino-N-sec-butyl-N-(2,2-dimethoxyethyl)acetamide (3.6 g, 16.5 mmol) in dichloromethane (40 mL) were added DIPEA (31.91 mL, 49.5 mmol), 3-(((9H-fluoren-9-yl)methoxy)carbonylamino)propanoic acid (5.14 g, 16.5 mmol) and T_3P (39.13 g, 33 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 16 hours. Progress of the reaction was monitored by TLC. After completion of the reaction, the reaction water (100 mL) was added and the organic phase was separated. The aqueous phase was extracted with dichloromethane (2 × 150 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography using silica (230-400 mesh; 23-25% ethyl acetate/petroleum ether as eluent). Collected pure fractions were concentrated under reduced pressure to afford (9H-fluoren-9-yl)methyl-3-(2-(sec-butyl(2,2-dimethoxyethyl)amino)-2-oxoethylamino)-3-oxopropylcarbamate (4.1 g, 48.6%) as a gum.

[0218] Step 4: Synthesis of (9H-fluoren-9-yl)methyl 8-sec-butyl-4,7-dioxooctahydro-1H-pyrazino[1,2-a]pyrimidine-1-carboxylate. To a solution of (9H-fluoren-9-yl)methyl-3-(2-(sec-butyl(2,2-dimethoxyethyl)amino)-2-oxoethylamino)-3-oxopropylcarbamate (4.1 g, 8.01 mmol) in acetic acid (2 mL) was stirred for 16 hours at room temperature. Progress of the reaction was

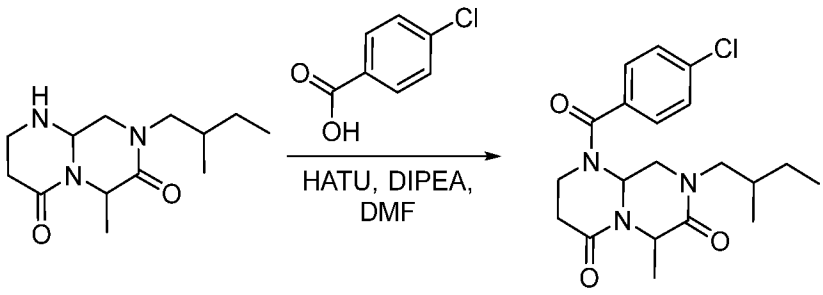
monitored by TLC. After TLC indicated complete consumption of the starting material, the reaction mixture was concentrated and the resulting mass was diluted with water and extracted with dichloromethane (2 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the product (9H-fluoren-9-yl)methyl 8-sec-butyl-4,7-dioxooctahydro-1H-pyrazino[1,2-a]pyrimidine-1-carboxylate. (3.2 g, 89.3%) as a gum.

[0219] Step 5: Synthesis of 8-sec-butyltetrahydro-1H-pyrazino[1,2-a]pyrimidine-4,7(6H,8H)-dione. To a solution of (9H-fluoren-9-yl)methyl 8-sec-butyl-4,7-dioxooctahydro-1H-pyrazino[1,2-a]pyrimidine-1-carboxylate (3.2 g, 7.1 mmol) in DMF (20 mL), cooled to 0 °C, was added piperidine (0.7 mL, 1.0 eq) and the reaction mixture was stirred at room temperature for 2 hours. Progress of the reaction was monitored by TLC. After TLC indicated complete consumption of starting material, the reaction mixture was washed with petroleum ether (2 × 50 mL) to remove the non-polar impurities. Cold water was added and extracted with dichloromethane (2 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the pure product (8-(sec-butyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (900 mg, 55.9%) as a solid.

[0220] Step 6: Synthesis of Compound 9. To a stirred solution of (8-(sec-butyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (0.500 g, 2.2 mmol) and 4-hydroxybenzaldehyde (0.271 g, 2.2 mmol) in methanol (10 mL) was added acetic acid (0.27 mL, 2.0 eq.) and picoline borane (0.285 g, 2.6 mmol) at room temperature. The reaction mixture was stirred at room temperature for 48 hr. The reaction progress was monitored by TLC. After completion, the reaction mixture was quenched with ice cold water (10 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude product. The crude compound was analyzed by LC/MS. The crude LC/MS data showed 8.28% desired mass. The crude compound was purified by column chromatography over silica gel (100-200), and 50-70% ethyl acetate in petroleum ether eluted the desired compound. The LC/MS of the eluted fractions showed 72.16% desired mass, which was further purified by Prep HPLC. After Prep HPLC purification, the fractions were collected and concentrated under reduced pressure, then lyophilized to afford Compound **9** (0.168 g, 22.8%) as a solid. Prep HPLC Method: Mobile Phase A: 10mM ammonium bicarbonate in water; Mobile Phase B: acetonitrile; Column: X-BRIDGE C_{18} (150 × 19mm 5μ); Flow: 18 mL/min. MS (ESI) m/z $[M+H]^+$: 332.2.

Example S11. Synthesis of Compound 10. The synthetic route for preparing Compound **10** is shown in Scheme 11.

Scheme 11.

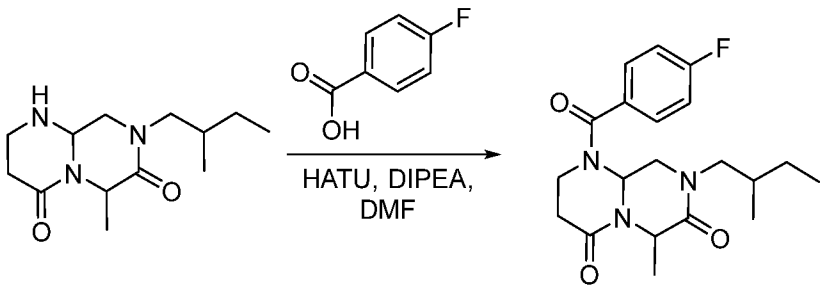


10

[0221] To a solution of 6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (250 mg, 0.98 mmol) and 4-chlorobenzoic acid (170 mg, 1.09 mmol) in DMF (4mL) at 0 °C was added HATU (413mg, 1.08mmol) followed by DIPEA (0.35mL, 1.97mmol). The reaction mixture was stirred at room temperature for 12 h. After completion, the reaction mixture was quenched with ice cold water (50 mL) and the aqueous layer was extracted with EtOAc (50 mL x 2). The organic layer was washed with cold H_2O (30 mL) followed by saturated brine (30 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200 mesh; 30% EtOAc in hexanes) to afford Compound **10** (1-(4-chlorobenzoyl)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione) (150 mg, 0.383 mmol, 39.2% yield) as a solid. MS (ESI) m/z $[M+H]^+$: 392.05. ^1H NMR (400 MHz, DMSO-d_6) δ 0.66 - 0.89 (m, 6 H) 0.91 - 1.42 (m, 4 H) 1.57 - 1.78 (m, 1 H) 2.16 - 2.35 (m, 2 H) 2.55-2.65 (m, 2 H) 3.08-3.23 (m, 2 H) 3.28-3.40 (m, 1 H) 3.51-3.64 (m, 2 H) 4.76-4.89 (m, 1 H) 5.88-6.02 (m, 1 H) 7.46-7.56 (m, 4 H).

Example S12. Synthesis of Compound 11. The synthetic route for preparing Compound **11** is shown in Scheme 12.

Scheme 12.



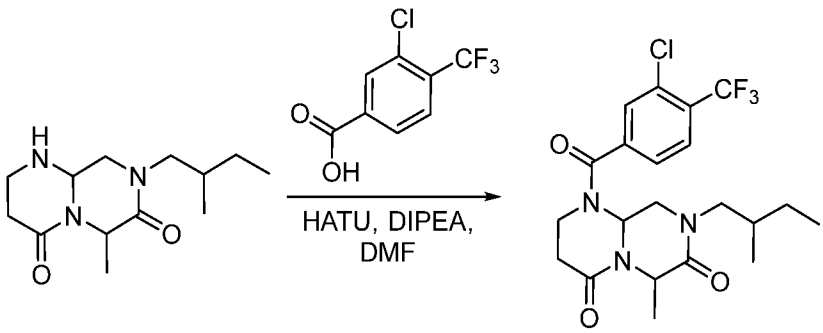
11

[0222] To a solution of 6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (250mg, 0.98mmol) and 4-fluorobenzoic acid (153 mg, 1.09 mmol) in DMF (4 mL) at 0°C was added HATU (413mg, 1.08mmol) followed by DIPEA (0.35 mL, 1.97mmol). The reaction mixture was stirred at room temperature for 12 h. After completion, the reaction mixture was quenched with ice cold water (50 mL) and the aqueous layer was extracted

with EtOAc (50 mL x 2). The organic layer was washed with cold H_2O (30 mL) followed by saturated brine (30 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200 mesh; 30% EtOAc in hexanes) to afford Compound **11** (1-(4-fluorobenzoyl)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione) (140 mg, 0.37 mmol, 38.0% yield) as a solid. MS (ESI) m/z $[M+H]^+$: 376.05. ^1H NMR (400 MHz, DMSO-d6) δ 0.69 - 0.81 (m, 3 H) 0.86 (t, J=7.23 Hz, 3 H) 0.95 - 1.14 (m, 2 H) 1.20 - 1.43 (m, 4 H) 1.59 - 1.80 (m, 2 H) 2.26 (d, J=16.95 Hz, 1 H) 2.55 - 2.72 (m, 1 H) 3.20 - 3.31 (m, 2 H) 3.35 - 3.39 (m, 1 H) 3.52 - 3.70 (m, 2 H) 4.73 - 4.89 (m, 1 H) 7.33 (t, J=8.73 Hz, 2 H) 7.61 (dd, J=8.23, 5.73 Hz, 2 H).

Example S13. Synthesis of Compound 12. The synthetic route for preparing Compound **12** is shown in Scheme 13.

Scheme 13.

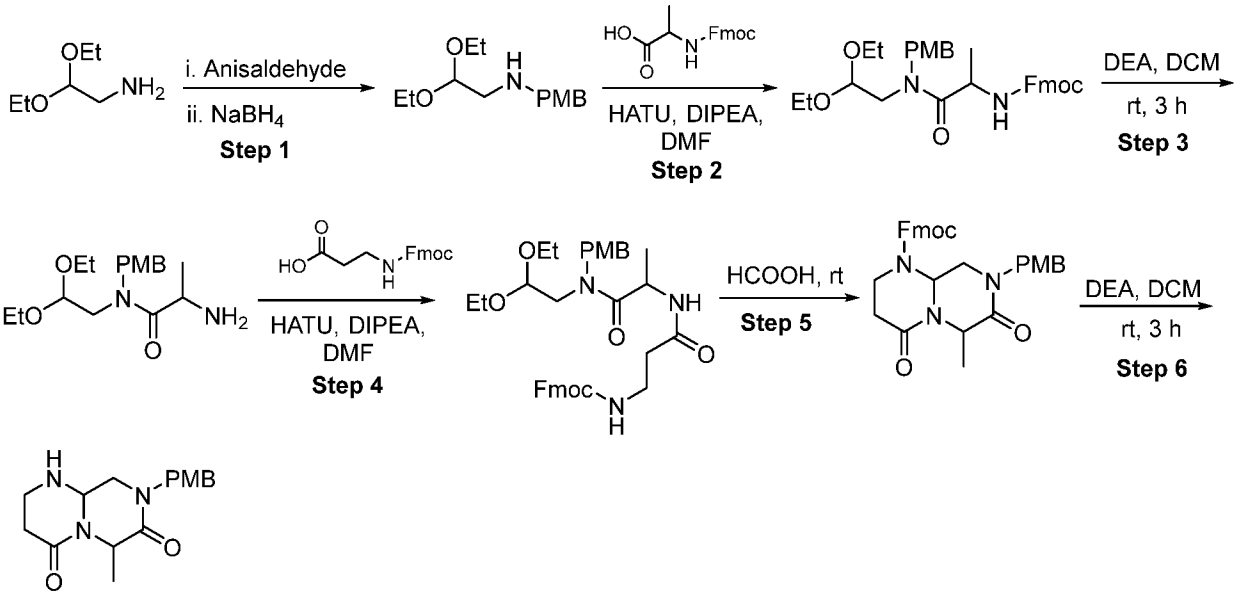


12

[0223] To a solution of 6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (250 mg, 0.98 mmol) and 3-chloro-4-(trifluoromethyl)benzoic acid (242 mg, 1.09 mmol) in DMF (4mL) at 0 °C was added HATU (413 mg, 1.08 mmol) followed by DIPEA (0.35mL, 1.97mmol). The reaction mixture was stirred at room temperature for 12 h. After completion, the reaction mixture was quenched with ice cold water (50 mL) and the aqueous layer was extracted with EtOAc (50 mL x 2). The organic layer was washed with cold H_2O (30 mL) followed by saturated brine (30 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200 mesh; 30% EtOAc in hexanes) to afford Compound **12** (1-(3-chloro-4-(trifluoromethyl)benzoyl)-6-methyl-8-(2-methylbutyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione) (250 mg, 0.55 mmol, 55.2% yield) as a solid. MS (ESI) m/z $[M+H]^+$: 460.0. ^1H NMR (400 MHz, DMSO-d6) δ 0.74 - 0.93 (m, 6 H) 0.98 - 1.19 (m, 2 H) 1.28 - 1.46 (m, 3 H) 1.64 - 1.81 (m, 1 H) 2.22 (d, J=17.45 Hz, 1 H) 2.57 - 2.70 (m, 1 H) 3.14 (dd, J=13.21, 6.23 Hz, 1 H) 3.25 - 3.31 (m, 2 H) 3.44 - 3.57 (m, 1 H) 3.61 - 3.87 (m, 2 H) 4.78 - 4.90 (m, 1 H) 5.89 - 6.05 (m, 1 H) 7.72 (d, J=7.98 Hz, 1 H) 7.90 - 8.02 (m, 2 H).

Example S14. Synthesis of Intermediate Compound 8-(4-methoxybenzyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione. The synthetic route for preparing this intermediate compound is shown in Scheme 14.

Scheme 14.



[0224] Step 1: Synthesis of 2,2-diethoxy-N-(4-methoxybenzyl)ethan-1-amine. A 500 mL round bottom flask was charged with anisaldehyde (12 mL, 90.22 mmol) and 2,2-diethoxyethanamine (10 g, 75.18 mmol). The reaction mixture was heated at 100 °C for 1 h. The reaction mixture was allowed to cool at room temperature and to this was added EtOH (100 mL) followed by $NaBH_4$ (4.28 g, 112.7 mmol). The resulting reaction mixture was stirred at room temperature for 16 h. After complete consumption of starting material (monitored by TLC), the reaction mixture was concentrated under reduced vacuum. The crude obtained was dissolved in EtOAc (300 mL). The organic layer was washed with brine (100 mL), dried over Na_2SO_4 and concentrated under vacuum to give crude product. The crude product obtained was purified by column chromatography (silica 100-200 mesh; 70% EtOAc in hexanes) to obtain 2,2-diethoxy-N-(4-methoxybenzyl)ethan-1-amine (15 g, 59.28 mmol,78% yield) as a liquid. MS (ESI) m/z $[M+H]^+$: 254.3.

[0225] Step 2: (9H-fluoren-9-yl)methyl (1-((2,2-diethoxyethyl)(4-methoxybenzyl)amino)-1-oxopropan-2-yl)carbamate. To a stirred solution of (((9H-fluoren-9-yl)methoxy)carbonyl)alanine (32 g, 102.76 mmol) in dry DMF (140 mL) maintained at 0 ºC was added HATU (42 g, 110.67 mmol), DIPEA (21.06 mL, 118.57 mmol), followed by 2,2-diethoxy-N-(4-methoxybenzyl)ethan-1-amine (20 g, 79.05 mmol). The reaction mixture was stirred at room temperature for 16 h. After complete consumption of starting material, the reaction mixture was quenched with ice cold water (300 mL) and the aqueous layer was extracted with EtOAc (200 mL x 2). The organic layer was washed with cold H_2O (200 mL) followed by brine

(100mL), dried over Na_2SO_4 and concentrated under reduced pressure to give crude product. The crude obtained was purified by column chromatography (Silica 100-200 mesh; 50% EtOAc in hexanes) to afford (9H-fluoren-9-yl)methyl (1-((2,2-diethoxyethyl)(4-methoxybenzyl)amino)-1-oxopropan-2-yl)carbamate (28 g, 51.22 mmol, 64.8% yield) as a gummy liquid. MS (ESI) m/z $[M+H-EtOH]^+$: 501.2.

[0226] Step 3: Synthesis of 2-amino-N-(2,2-diethoxyethyl)-N-(4-methoxybenzyl)propanamide. To a solution of (9H-fluoren-9-yl) methyl (1-((2,2-diethoxyethyl)(4-methoxybenzyl)amino)-1-oxopropan-2-yl)carbamate (28 g, 51.22 mmol) in CH_2Cl_2 (30 mL) was added diethylamine (200 mL). The reaction mixture was stirred at room temperature for 3 h. After complete consumption of the starting material (monitored by TLC), the reaction mixture was concentrated and the crude obtained was purified by column chromatography (Silica 100-200mesh; 5% MeOH in DCM) to afford 2-amino-N-(2,2-diethoxyethyl)-N-(4-methoxybenzyl)propanamide (14.5 g, 44.75 mmol, 87% yield) as a viscous liquid. MS (ESI) m/z $[M+H-EtOH]^+$: 279.05.

[0227] Step 4: Synthesis of (9H-fluoren-9-yl)methyl (3-((1-((2,2-diethoxyethyl)(4-methoxybenzyl)amino)-1-oxopropan-2-yl)amino)-3-oxopropyl)carbamate. To a stirred solution of 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid (14.78 g, 47.53 mmol) in dry DMF (120 mL) maintained at 0°C was added HATU (18.06 g, 47.53 mmol), DIPEA (9.21 mL, 51.85 mmol) followed by 2-amino-N-(2,2-diethoxyethyl)-N-(4-methoxybenzyl)propanamide (14 g, 43.20 mmol). The reaction mixture was stirred for 16 h at room temperature. After completion, the reaction mixture was quenched with ice cold water (200 mL) and the aqueous layer was extracted with EtOAc (200 mL x 2). The organic layer was washed with cold H_2O (500 mL) followed by saturated brine (200 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200 mesh; 30% EtOAc in hexanes) to afford (9H-fluoren-9-yl)methyl (3-((1-((2,2-diethoxyethyl)(4-methoxybenzyl)amino)-1-oxopropan-2-yl)amino)-3-oxopropyl)carbamate (18 g, 29.14 mmol, 67.44 % yield) as a viscous liquid. MS (ESI) m/z $[M+H-EtOH]^+$: 572.

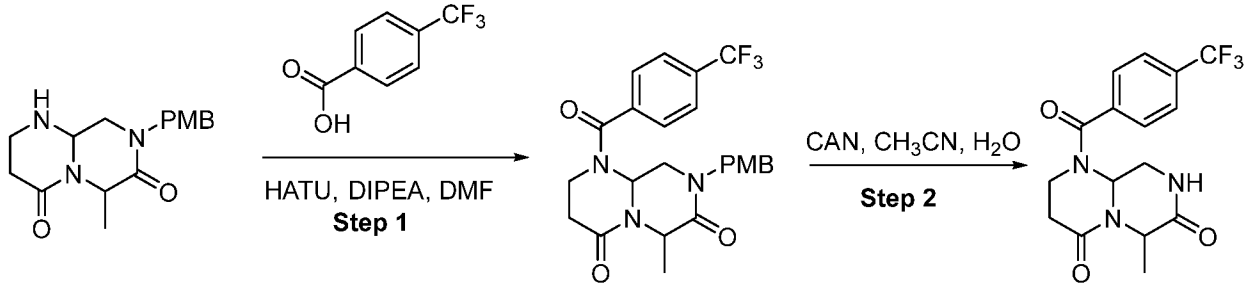
[0228] Step 5: Synthesis of (9H-fluoren-9-yl)methyl 8-(4-methoxybenzyl)-6-methyl-4,7-dioxohexahydro-2H-pyrazino[1,2-a]pyrimidine-1(6H)-carboxylate. A solution of (9H-fluoren-9-yl)methyl (3-((1-((2,2-diethoxyethyl)(4-methoxybenzyl)amino)-1-oxopropan-2-yl)amino)-3-oxopropyl)carbamate (18 g, 29.14 mmol) in formic acid (120 mL) was stirred at room temperature for 12 h. After completion, the reaction mixture was concentrated and the crude obtained was purified by column chromatography (Silica 100-200 mesh; 30% EtOAc in hexanes) to afford (9H-fluoren-9-yl)methyl 8-(4-methoxybenzyl)-6-methyl-4,7-dioxohexahydro-

2H-pyrazino[1,2-a]pyrimidine-1(6H)-carboxylate (14.5 g, 27.58 mmol, 94% yield) as a solid. MS (ESI) m/z $[M+H]^+$: 526.

[0229] Step 6: Synthesis of 8-(4-methoxybenzyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione. To a solution of (9H-fluoren-9-yl)methyl 8-(4-methoxybenzyl)-6-methyl-4,7-dioxohexahydro-2H-pyrazino[1,2-a]pyrimidine-1(6H)-carboxylate (14 g, 26.63 mmol) in CH_2Cl_2 (150 mL) was added diethyl amine (100 mL) and the reaction mixture was stirred at room temperature for 3 h. After complete consumption of the starting material (monitored by TLC), the reaction mixture was concentrated and the crude obtained was purified by column chromatography (Silica 100-200 mesh; 5% MeOH in DCM) to afford 8-(4-methoxybenzyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (7 g, 23.07 mmol, 87 % yield) as a sticky solid. MS (ESI) m/z $[M+H]^+$: 304.

Example S15. Synthesis of Intermediate Compound 8-(4-methoxybenzyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione. The synthetic route for preparing this intermediate compound is shown in Scheme 15.

Scheme 15.



[0230] Step 1: Synthesis of 8-(4-methoxybenzyl)-6-methyl-1-(4-(trifluoromethyl)benzoyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione. To a solution of 4-(trifluoromethyl)benzoic acid (5.26 g, 27.69 mmol) in DMF (100 mL) maintained at 0 °C was added HATU (10.52 g, 27.69 mmol), DIPEA (12.30 mL, 69.23 mmol) followed by 8-(4-methoxybenzyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (7 g, 23.07 mmol), and the reaction mixture was stirred at room temperature for 12 h. After completion, the reaction mixture was quenched with ice cold water (200 mL) and the aqueous layer was extracted with EtOAc (200 mL x 2). The organic layer was washed with cold H_2O (200 mL) followed by saturated brine (150mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200 mesh; 30% EtOAc in hexanes) to afford 8-(4-methoxybenzyl)-6-methyl-1-(4-(trifluoromethyl)benzoyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (9 g, 18.92 mmol, 82.04 % yield) as a solid. MS (ESI) m/z $[M+H]^+$: 476.15 and MS (ESI) m/z $[M+Na]^+$: 498.05.

[0231] Step 2: Synthesis of 6-methyl-1-(4-(trifluoromethyl)benzoyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione. To a solution of 8-(4-methoxybenzyl)-6-methyl-1-(4-(trifluoromethyl)benzoyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (9 g, 18.92 mmol) in CH_3CN:H_2O (2:1, 150 mL) maintained at 0°C, was added CAN (31.15 g, 56.82 mmol) and the reaction mixture was allowed to stir at room temperature for 3 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was quenched with saturated solution of aq. $NaHCO_3$ (200 mL) and extracted with EtOAc (200 mL×2). The combined organic layer was washed with H_2O (200 mL) followed by saturated brine solution (150 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200 mesh; 10% MeOH in DCM) to afford 6-methyl-1-(4-(trifluoromethyl)benzoyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (3.5 g, 9.85 mmol, 52.8% yield) as a solid. MS (ESI) m/z $[M+H+CH_3CN]^+$: 397.0. ^1H NMR (400 MHz, DMSO-d6) δ 1.25 - 1.46 (m, 3 H) 2.15-2.30 (m, 1 H) 2.56 - 2.69 (m, 1 H) 3.16 (d, J=4.99 Hz, 1 H) 3.22-3.30 (m, 1 H) 3.42 - 3.72 (m, 2 H) 4.70 - 4.87 (m, 1 H) 5.85-5.95 (m, 1 H) 7.75 (d, J=7.98 Hz, 2 H) 7.86 (d, J=7.98 Hz, 2 H) 8.11 (brs, 1 H).

Example S16. General Procedure A for the Synthesis of Final Compounds.

[0232] To a solution of 6-methyl-1-(4-(trifluoromethyl)benzoyl)hexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (200 mg,0.56 mmol) in DMF (2 mL) was added KO^tBu (1M in THF,1.69 mmol, 1.69 mL) followed by the appropriate alkyl halide (1.12 mmol), and the reaction mixture was exposed to microwave irradiation at 120°C for 1 h. The reaction mixture was cooled to room temperature and quenched with H_2O (25 mL). The aqueous layer was extracted with EtOAc (10 mL×3). The combined organic layers were washed with brine and concentrated. The crude product obtained was purified by CombiFlash.

Example S17. Synthesis of Compound 15.

[0233] Compound **15** was synthesized by General Procedure A using (bromomethyl)cyclopentane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 438.65. ^1H NMR (400 MHz, DMSO-d_6) δ 1.02 - 1.26 (m, 3 H) 1.28 - 1.42 (m, 2 H) 1.44 - 1.76 (m, 6 H) 1.80 - 2.08 - 2.33 (m, 2 H) 2.55 - 2.71 (m, 1 H) 3.22 (dd, *J*=12.96, 7.48 Hz, 1 H) 3.26 - 3.32 (m, 1 H) 3.39 (d, *J*=6.98 Hz, 1 H) 3.49-3.57 (m, 1 H) 3.59-3.74 (m, 1 H) 3.76-3.91 (m, 1 H) 4.80-4.90 (m, 1 H) 5.95-6.05 (m, 1 H) 7.72 - 7.79 (m, 2 H) 7.84 - 7.91 (m, 2 H).

Example S18. Synthesis of Compound 16.

[0234] Compound **16** was synthesized by General Procedure A using bromomethylcyclobutane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 424.15. ^1H NMR (400 MHz, DMSO-d_6) δ 1.29 - 1.44 (m, 2 H) 1.58 - 1.89 (m, 4 H) 1.90 - 2.08 (m, 2 H) 2.16-2.31 (m, 1 H) 2.55 - 2.70 (m, 2 H) 3.18 - 3.31 (m, 1 H) 3.25 - 3.26 (m, 1 H) 3.34 - 3.42 (m, 1 H) 3.36 -

3.57 (m, 2 H) 3.60-3.69 (n, 1 H) 3.71-3.83 (m, 1 H) 4.75-4.89 (m, 1 H) 5.90-6.05 (m, 1 H) 7.70 - 7.79 (m, 2 H) 7.87 (d, *J*=8.31 Hz, 2 H).

Example S19. Synthesis of Compound 19.

[0235] Compound **19** was synthesized by General Procedure A using (2-bromoethyl)cyclopentane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 452.35. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 - 1.18 (m, 3 H) 1.26 - 1.61 (m, 9 H) 1.66-1.83 (m, 2 H) 2.16-2.31 (m, 1 H) 2.56 - 2.70 (m, 1 H) 3.16 - 3.28 (m, 1 H) 3.35 - 3.56 (m, 3 H) 3.60-3.73 (m, 1 H) 3.77-3.90 (m, 1 H) 4.72 - 4.92 (m, 1 H) 5.94-6.06 (m, 1 H) 7.77 (d, *J*=7.98 Hz, 2 H) 7.87 (d, *J*=7.98 Hz, 2 H).

Example S20. Synthesis of Compound 20.

[0236] Compound **20** was synthesized by General Procedure A using (2-bromoethyl)cyclobutane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 438.25. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.27 - 1.44 (m, 3 H) 1.50-1.71 (m, 4 H) 1.71-1.88 (m, 2 H) 1.93 - 2.09 (m, 2 H) 2.13 - 2.34 (m, 2 H) 2.56 - 2.70 (m, 2 H) 3.25 - 3.32 (m, 1 H) 3.35 - 3.42 (m, 1 H) 3.45-3.55 (m, 1 H) 3.59 - 3.72 (m, 1 H) 3.74-3.90 (m, 1 H) 4.75-4.89 (m, 1 H) 5.94-6.05 (m, 1 H) 7.71 - 7.79 (m, 2 H) 7.87 (d, *J*=8.31 Hz, 2 H).

Example S21. Synthesis of Compound 21.

[0237] Compound **21** was synthesized by General Procedure A using 1-bromobutane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 412.20. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81-0.97 (m, 3 H) 1.15 - 1.57 (m, 7 H) 2.15-2.31 (m, 1 H) 2.57 - 2.69 (m, 1 H) 3.14 - 3.28 (m, 1 H) 3.35 - 3.60 (m, 3 H) 3.62-3.73 (m, 1 H) 3.74 - 3.92 (m, 1 H) 4.75 - 4.91 (m, 1 H) 5.94-6.06 (m, 1 H) 7.76 (d, *J*=7.34 Hz, 2 H) 7.87 (d, *J*=7.83 Hz, 2 H).

Example S22. Synthesis of Compound 22.

[0238] Compound **22** was synthesized by General Procedure A using 4-bromobut-1-ene as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 410.20. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.28-1.45 (m, 3 H) 2.14-2.38 (m, 3 H) 2.55 - 2.69 (m, 1 H) 3.36 - 3.57 (m, 4 H) 3.58-3.72 (m, 1 H) 3.75-3.89 (m, 1 H) 4.75 - 4.90 (m, 1 H) 4.98 - 5.19 (m, 2 H) 5.69-5.84 (m, 1 H) 5.93-6.05 (m, 1 H) 7.76 (d, *J*=7.98 Hz, 2 H) 7.88 (d, *J*=7.98 Hz, 2 H).

Example S23. Synthesis of Compound 23.

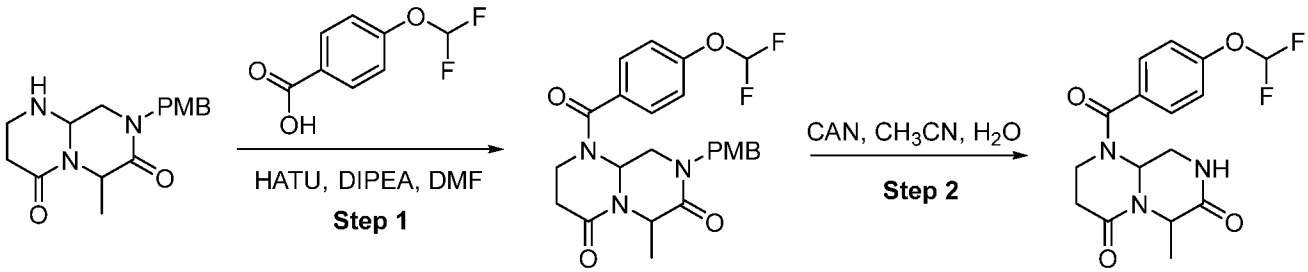
[0239] Compound **23** was synthesized by General Procedure A using 1-bromo-2-methylpropane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 412.25. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.80-0.96 (m, 6 H) 1.30 - 1.48 (m, 3 H) 1.85-2.03 (m, 1 H) 2.15-2.31 (m, 1 H) 2.57 - 2.70 (m, 1 H) 3.06-3.16 (m, 1 H) 3.18-3.28 (m, 1 H) 3.36-3.45 (m, 1 H) 3.44 - 3.57 (m, 1 H) 3.60-3.74 (m, 1 H) 3.73 - 3.87 (m, 1 H) 4.77-4.92 (m, 1 H) 5.93-6.07 (m, 1 H) 7.76 (d, *J*=7.48 Hz, 2 H) 7.87 (d, *J*=7.48 Hz, 2 H).

Example S24. Synthesis of Compound 24.

[0240] Compound **24** was synthesized by General Procedure A using 2-bromopropane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 398.55. ^1H NMR (400 MHz, DMSO-d_6) δ 1.10 (d, J=5.49 Hz, 6 H) 1.28-1.45 (m, 3 H) 2.16-2.24 (m, 1 H) 2.56 - 2.71 (m, 1 H) 3.34-3.40 (m, 1 H) 3.44 - 3.79 (m, 3 H) 4.59-4.72 (m, 1 H) 4.75-4.90 (m, 1 H) 5.86-6.00 (m, 1 H) 7.79 (d, J=7.98 Hz, 2 H) 7.83 - 7.92 (m, 2 H).

Example S25. Synthesis of Intermediate Compound 1-(4-(difluoromethoxy)benzoyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione. The synthetic route for preparing this intermediate compound is shown in Scheme 16.

Scheme 16.



[0241] Step 1: Synthesis of 1-(4-(difluoromethoxy)benzoyl)-8-(4-methoxybenzyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione. To a solution of 4-(difluoromethoxy)benzoic acid (1.71 g, 9.08 mmol) in DMF (25 mL) maintained at 0 °C was added HATU (3.45g, 9.08mmol), DIPEA (4.34mL, 24.8mmol) followed by 8-(4-methoxybenzyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (2.5 g, 8.25 mmol) and reaction mixture was stirred at room temperature for 12 h. After completion, the reaction mixture was quenched with ice cold water (50 mL) and the aqueous layer was extracted with EtOAc (100 mL x 2). The organic layer was washed with cold H_2O (100 mL) followed by saturated brine (100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200 mesh; 30% EtOAc in hexanes) to afford 1-(4-(difluoromethoxy)benzoyl)-8-(4-methoxybenzyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (3.5 g, 7.38 mmol, 89.5% yield) as a solid. MS (ESI) m/z $[M+H]^+$: 474.12.

[0242] Step 2: Synthesis of 1-(4-(difluoromethoxy)benzoyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione. To a solution of 1-(4-(difluoromethoxy)benzoyl)-8-(4-methoxybenzyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (3.0 g, 6.34 mmol) in CH_3CN:H_2O (2:1, 45 mL) maintained at 0 °C, was added CAN (12.0 g, 21.90 mmol) and the reaction mixture was allowed to stir at room temperature for 3 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was quenched with saturated solution of aq. $NaHCO_3$ (100 mL) and extracted with EtOAc (200 mL×2). The

combined organic layer was washed with H_2O (250 mL) followed by saturated brine solution (250 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200 mesh; 10% MeOH in DCM) to afford 1-(4-(difluoromethoxy)benzoyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (2.0 g, 5.66 mmol, 89.6% yield) as a solid. MS (ESI) m/z $[M+H]^+$: 353.95. ^1H NMR (400 MHz, DMSO-d_6) δ 1.10 - 1.39 (m, 3 H) 2.17-2.18 (m, 1 H) 2.52 - 2.68 (m, 1 H) 3.18 - 3.27 (m, 2 H) 3.44 - 3.71 (m, 2 H) 4.69 - 4.83 (m, 1 H) 5.75 - 5.92 (m, 1 H) 7.24 (d, *J*=7.83 Hz, 2 H) 7.32 (t, *J*=72.0 Hz, 1 H) 7.57 (d, *J*=8.31 Hz, 2 H) 8.04 (brs, 1 H).

Example S26. General Procedure B for the Synthesis of Final Compounds.

[0243] To a solution of 1-(4-(difluoromethoxy)benzoyl)-6-methylhexahydro-4H-pyrazino[1,2-a]pyrimidine-4,7(6H)-dione (200 mg, 0.56 mmol) in DMF (4 mL) maintained at 0°C was added NaH (122 mg, 2.8 mmol, 55% dispersion in mineral oil) and the reaction mixture was stirred at the same temperature for 15 minutes. To this reaction mixture was added the appropriate alkyl halide (1.6 mmol) and the reaction mixture was allowed to warm to room temperature and stirred for 3 h. After completion, the reaction mixture was quenched with ice cold H_2O (15 mL) and aqueous layer was extracted with EtOAc (15 mL×3). The combined organic layer was washed with brine and concentrated. The crude product obtained was purified by CombiFlash.

Example S27. Synthesis of Compound 13.

[0244] Compound **13** was synthesized by General Procedure B using (bromomethyl)cyclopentane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 436.05. ^1H NMR (400 MHz, DMSO-d_6) δ 1.07-1.16 (m, 3 H) 1.32 (d, J=6.48 Hz, 3 H) 1.41 - 1.73 (m, 7 H) 2.06 - 2.21 (m, 1 H) 2.21 - 2.34 (m, 1 H) 2.54 - 2.70 (m, 1 H) 3.14 - 3.29 (m, 1 H) 3.35 - 3.45 (m, 1 H) 3.52 - 3.69 (m, 1 H) 3.75 - 3.93 (m, 1 H) 4.75 - 4.91 (m, 1 H) 5.88-5.99 (m, 1 H) 7.27 (d, J=8.48 Hz, 2 H) 7.35 (t, J=72.0 Hz, 1 H) 7.61 (d, J=8.98 Hz, 2 H).

Example S28. Synthesis of Compound 14.

[0245] Compound **14** was synthesized by General Procedure B using (bromomethyl)cyclobutane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 421.14. ^1H NMR (400 MHz, DMSO-d_6) δ 1.16-1.25 (m, 1 H) 1.27-1.43 (m, 3 H) 1.54 - 1.73 (m, 2 H) 1.73 - 1.86 (m, 2 H) 1.89-2.03 (m, 2 H) 2.24 (d, *J*=17.12 Hz, 1 H) 2.53 - 2.69 (m, 2 H) 3.20-3.28 (m, 1 H) 3.29-3.40 (m, 1 H) 3.40 - 3.66 (m, 2 H) 3.69 - 3.87 (m, 1 H) 4.75-4.86 (m, 1 H) 5.74 - 6.02 (m, 1 H) 7.26 (d, *J*=8.31 Hz, 2 H)) 7.33 (t, *J*=72.0 Hz, 1 H) 7.59 (d, *J*=8.31 Hz, 2 H).

Example S29. Synthesis of Compound 17.

[0246] Compound **20** was synthesized by General Procedure B using 1-bromobutane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 410.0. ^1H NMR (400 MHz, DMSO-d_6) δ 0.81 - 0.96 (m, 3

H) 1.15 - 1.39 (m, 4 H) 1.40-1.55 (m, 2 H) 2.26 (d, *J*=16.95 Hz, 1 H) 2.53 - 2.70 (m, 2 H) 3.12 - 3.30 (m, 2 H) 3.38 - 3.46 (m, 1 H) 3.56-3.74 (m, 2 H) 3.75-3.92 (m, 1 H) 4.84 (q, *J*=6.81 Hz, 1 H) 5.86-6.06 (m, 1 H) 7.28 (d, *J*=7.98 Hz, 2 H) 7.36 (t, *J*=72.0 Hz, 1 H) 7.62 (d, *J*=8.48 Hz, 2 H).

Example S30. Synthesis of Compound 18.

[0247] Compound **18** was synthesized by General Procedure B using 4-bromobut-1-ene as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 408.06. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.45 (m, 3 H) 2.18 - 2.33 (m, 3 H) 2.53 - 2.70 (m, 1 H) 3.36 - 3.46 (m, 3 H) 3.51 - 3.72 (m, 2 H) 3.74-3.90 (m, 1 H) 4.84 (q, *J*=6.65 Hz, 1 H) 4.91-5.15 (m, 2 H) 5.67-5.84 (m, 1 H) 5.86 - 6.03 (m, 1 H) 7.29 (d, *J*=8.48 Hz, 2 H) 7.36 (t, *J*=72.0 Hz, 1 H) 7.61 (d, *J*=8.48 Hz, 2 H).

Example S31. Synthesis of Compound 27.

[0248] Compound **27** was synthesized by General Procedure B using 2-(bromomethyl)tetrahydrofuran as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 438.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.55 (m, 2 H), 7.20 – 7.30 (m, 2 H), 6.40 – 6.76 (m, 1 H), 5.90 - 6.20 (m, 1 H), 5.16 - 5.26 (m, 1 H), 4.06 – 4.17 (m, 2H), 3.82 - 3.92 (m, 4 H), 3.61 – 3.77 (m, 2 H), 2.83 – 2.99 (m, 1 H), 2.47 – 2.59 (m, 2 H), 2.01 – 2.12 (m, 4 H), 1.49 (s, 3 H).

Example S32. Synthesis of Compound 28.

[0249] Compound **28** was synthesized by General Procedure B using (2-bromoethyl)benzene as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 458.10. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.50 (m, 2 H), 7.20 – 7.28 (m, 2 H), 7.33 – 7.43 (m, 5 H), 6.40 – 6.76 (m, 1 H), 5.90 - 6.20 (m, 1 H), 5.16 - 5.26 (m, 1 H), 3.72 – 3.96 (m, 2H), 3.44 - 3.52 (m, 1 H), 3.25 – 3.35 (m, 2 H), 2.83 – 2.99 (m, 2 H), 2.47 – 2.59 (m, 1 H), 2.42 – 2.60 (m, 1 H), 2.30 – 2.57 (m, 1H), 1.49 (s, 3 H).

Example S33. Synthesis of Compound 29.

[0250] Compound **29** was synthesized by General Procedure B using 4-(2-bromoethyl)pyridine as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 459.10. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 – 8.58 (m, 2 H), 7.24 – 7.46 (m, 4 H), 7.18 (d, J = 7.99Hz, 2 H), 6.40 – 6.76 (m, 1 H), 5.90 - 6.20 (m, 1 H), 5.16 - 5.26 (m, 1 H), 3.72 – 3.96 (m, 2H), 3.44 - 3.52 (m, 1 H), 3.25 – 3.35 (m, 2 H), 2.83 – 2.99 (m, 2 H), 2.47 – 2.59 (m, 1 H), 2.42 – 2.60 (m, 1 H), 2.30 – 2.57 (m, 1H), 1.49 (s, 3 H).

Example S34. Synthesis of Compound 30.

[0251] Compound **30** was synthesized by General Procedure B using (3-bromopropyl)cyclopropane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 459.10. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 – 8.58 (m, 2 H), 7.24 – 7.46 (m, 4 H), 7.18 (d, J = 7.99Hz, 2 H), 6.40 – 6.76 (m, 1 H), 5.90 - 6.20 (m, 1 H), 5.16 - 5.26 (m, 1 H), 3.72 – 3.96 (m, 2H), 3.44 - 3.52 (m, 1

H), 3.25 – 3.35 (m, 2 H), 2.83 – 2.99 (m, 2 H), 2.47 – 2.59 (m, 1 H), 2.42 – 2.60 (m, 1 H), 2.30 – 2.57 (m, 1H), 1.49 (s, 3 H).

Example S35. Synthesis of Compound 31.

[0252] Compound **31** was synthesized by General Procedure B using (2-bromoethyl)cyclopropane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 422.2. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J = 8.01 Hz, 2H), 7.20 – 7.28 (m, 2 H), 6.40 – 6.76 (m, 1 H), 5.90 - 6.20 (m, 1 H), 5.16 - 5.26 (m, 1 H), 3.72 – 3.96 (m, 1H), 3.46 - 3.64 (m, 5 H), 2.46 – 2.64 (m, 2 H), 1.43 – 1.56 (m, 5 H), 0.43-0.65 (m, 2H), 0.75-0.85 (m, 2 H).

Example S36. Synthesis of Compound 32.

[0253] Compound **32** was synthesized by General Procedure B using 1-bromo-2-methoxyethane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 412.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52-7.62 (m, 2 H), 7.16 – 7.34 (m, 3 H), 5.85 – 5.95 (m, 1 H), 4.80 – 4.90 (m, 1 H), 3.85 – 3.95 (m, 1 H), 3.70 – 3.80 (m, 2 H), 3.25 – 3.46 (m, 5H), 3.22 (s, 3 H), 2.62 – 2.72 (m, 1 H), 2.20 – 2.30 (m, 1 H), 1.49 (s, 3 H).

Example S37. Synthesis of Compound 33.

[0254] Compound **33** was synthesized by General Procedure B using 1-bromo-3-methoxypropane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 426.20. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52-7.62 (m, 2 H), 7.16 – 7.34 (m, 3 H), 5.85 – 5.95 (m, 1 H), 4.80 – 4.90 (m, 1 H), 3.85 – 3.95 (m, 1 H), 3.70 – 3.80 (m, 2 H), 3.58 – 3.68 (m, 2H), 3.45 – 3.55 (m, 4H), 3.22 (s, 3 H), 2.62 – 2.72 (m, 1 H), 2.20 – 2.30 (m, 2 H), 1.49 (s, 3 H).

Example S38. Synthesis of Compound 36.

[0255] Compound **36** was synthesized by General Procedure B using (2-bromoethyl)methylsulfone as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 459.95. ^{1}H NMR (400 MHz, CHLOROFORM) δ 7.49 (d, J = 8.01 Hz, 2 H), 7.15 - 7.26 (m, 2 H), 6.40 – 6.76 (m, 1 H), 5.90 – 6.20 (m, 1 H), 5.15 – 5.25 (m, 1 H), 3.86 – 3.97 (m, 3 H), 3.66 - 3.77 (m, 2 H), 3.38 - 3.49 (m, 3 H), 2.97 (s, 3 H), 2.59 – 2.69 (m, 2 H), 1.49 (s, 3 H).

Example S39. Synthesis of Compound 34.

[0256] Step 1. Synthesis of 8-(2-((tert-butyldimethylsilyl)oxy)ethyl)-1-(4-(difluoromethoxy)benzoyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione. To a solution of 1-(4-(difluoromethoxy)benzoyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (0.300 g, 0.849 mmol) in DMF (6 mL) was added Cs_2CO_3 (0.827 g, 2.547 mmol) followed by (2-bromoethoxy)(tert-butyl)dimethylsilane (0.243 g, 1.018 mmol) at 0 °C and the reaction mixture was heated at 120 °C in sealed tube for 1 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was slowly quenched with ice cold water (30 mL) and extracted with EtOAc (50 mL). The combined organic layer was washed

with ice cold brine solution (3 x 30 mL), dried over Na_2SO_4 and concentrated under reduced pressure to afford 8-(2-((tert-butyldimethylsilyl)oxy)ethyl)-1-(4-(difluoromethoxy)benzoyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (0.250 g, crude). The crude compound was as such used for next reaction without carried out further purification. MS (ESI) m/z $[M+H]^+$: 512.10.

[0257] Step 2. Synthesis of 1-(4-(difluoromethoxy)benzoyl)-8-(2-hydroxyethyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione. To a solution of 8-(2-((tert-butyldimethylsilyl)oxy)ethyl)-1-(4-(difluoromethoxy)benzoyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (0.250 g, 0.4886 mmol) in THF (5 mL) was added TBAF (3 mL) 0 °C temperature. The reaction mixture was allowed to attain room temperature and stirred for 6 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was slowly quenched with ice cold water (5 mL) and extracted with EtOAc (2 x 10 mL). The combined organic layer was washed with ice cold brine solution (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure to get crude compound. The crude compound obtained was purified by column chromatography (Silicagel 60-120 mesh; 10% MeOH in DCM) to afford Compound **34** (1-(4-(difluoromethoxy)benzoyl)-8-(2-hydroxyethyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione) (0.102 g,52% yield) white solid. MS (ESI) m/z $[M+H]^+$: 398.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52-7.62 (m, 2 H), 7.16 – 7.34 (m, 3 H), 5.92 – 6.02 (m, 1 H), 6.78 – 6.88 (m, 2 H), 3.86 – 3.92 (m, 1 H), 3.47 – 3.62 (m, 6 H), 3.21 – 3.31 (m, 1H), 2.57 – 2.67 (m, 1 H), 2.25 – 3.35 (m, 1 H), 1.49 (s, 3 H).

Example S40. Synthesis of Compound 35.

[0258] Step 1. Synthesis of 2-(1-(4-(difluoromethoxy)benzoyl)-6-methyl-4,7-dioxooctahydro-8*H*-pyrazino[1,2-*a*]pyrimidin-8-yl)acetonitrile. To a solution of 1-(4-(difluoromethoxy)benzoyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (0.300 g, 0.849 mmol) in DMF (6 mL) was added NaH (0.050 g, 1.274 mmol) followed by 2-bromoacetonitrile (0.112 g, 0.933 mmol) at 0 °C and the reaction mixture was allowed to stand for room temperature for 1 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was slowly quenched with ice cold water (70 mL) and extracted with EtOAc (100 mL). The combined organic layer was washed with ice cold brine solution (100 mL), dried over Na_2SO_4 and concentrated under reduced pressure to get crude compound. The crude compound obtained was purified by column chromatography (Silicagel 60-120 mesh; 10% MeOH in DCM) to afford 2-(1-(4-(difluoromethoxy)benzoyl)-6-methyl-4,7-dioxooctahydro-8*H*-pyrazino[1,2-*a*]pyrimidin-8-yl)acetonitrile (0.120 g, 36% yield) white solid. MS (ESI) m/z $[M+H]^+$: 393.05.

[0259] **Step 2. Synthesis of 8-(2-aminoethyl)-1-(4-(difluoromethoxy)benzoyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione.** To a solution of 2-(1-(4-(difluoromethoxy)benzoyl)-6-methyl-4,7-dioxooctahydro-8*H*-pyrazino[1,2-*a*]pyrimidin-8-yl)acetonitrile (0.120 g, 0.305 mmol) in ethanol (5 mL) was added Conc. HCl (0.100 mL) followed by Platinum oxide (0.012 g, 0.030 mmol) at room temperature and the reaction mixture was heated under Hydrogen gas atmosphere for 3 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was filtered through a pad of Celite. The Celite pad was washed with ethanol (20 mL) and filtrate was concentrated under reduced pressure to get crude compound. The crude compound was triturated with *n* pentane to afford Compound **35** (8-(2-aminoethyl)-1-(4-(difluoromethoxy)benzoyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione) (0.110 g, 90%) yield) white solid. MS (ESI) m/z $[M+H]^+$: 397.05. ^{1}H NMR (400 MHz, DMSO d_6) δ 7.96 (s, 2 H), 7.55 – 7.65 (m, 2 H), 7.20 – 7.35 (m, 3 H), 5.90 - 6.20 (m, 1 H), 4.85 – 4.95 (m, 1 H), 3.82 – 3.92 (m, 1H), 3.55. - 3.85 (m, 2 H), 3.35 – 3.45 (m, 3 H), 2.95 – 3.05 (m, 2 H), 2.60 – 2.70 (m, 1H), 2.20 – 2.30 (m, 1 H), 1.35 (s, 3 H).

Example S41. General Procedure C for the Synthesis of Final Compounds.

[0260] To a solution of 1-(4-(difluoromethoxy)benzoyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (0.200 g, 0.566 mmol) in DMF (5 mL) was added Cs_2CO_3 (0.735 g, 2.264 mmol, 4 eq) followed by the appropriate alkyl halide (0.679 mmol, 1.2 eq) at 0 °C and the reaction mixture was heated at 50 °C under microwave irradiation for 1 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was slowly quenched with ice cold water (6 mL) and extracted with EtOAc (20 mL×3). The combined organic layer was washed with saturated brine solution (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure to get crude compound. The crude compound obtained was purified by column chromatography to provide the final compound.

Example S42. Synthesis of Compound 25.

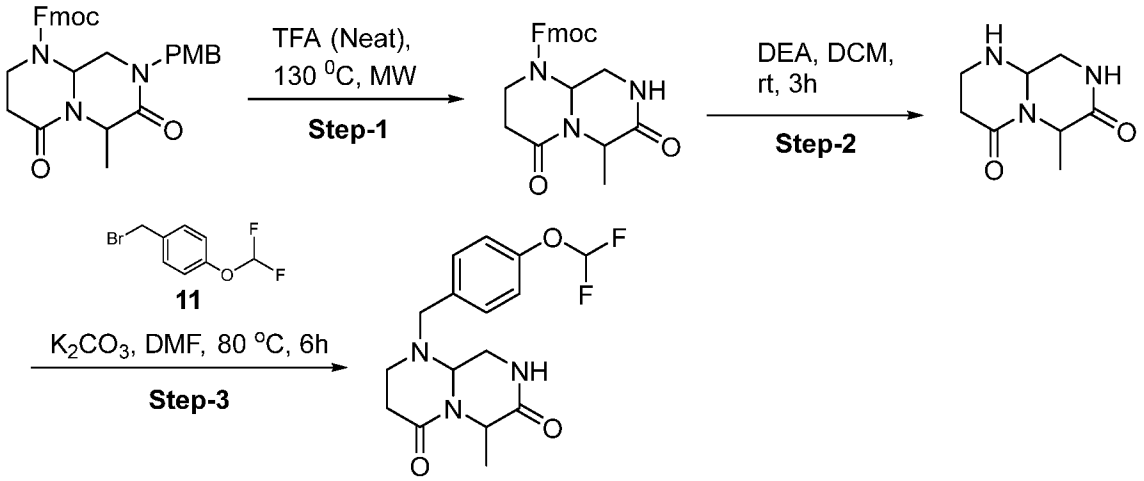
[0261] Compound **25** was synthesized by General Procedure C using 2-(2-iodoethyl)furan as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 448.10. ^{1}H NMR: δ 7.40 – 7.50 (m, 2 H), 7.28 – 7.38 (m, 1 H), 7.15-7.25 (m, 2 H), 6.39 – 6.78 (m, 1 H), 6.25-6.35 (m, 1 H), 5.90 - 6.12 (m, 2 H), 5.25 - 5.35 (m, 1 H), 5.10 – 5.20 (m, 1 H), 3.70 - 3.80 (m, 1 H), 3.50 - 3.60 (m, 1 H), 3.20 – 3.40 (m, 2 H), 2.95 – 3.05 (m, 3 H), 2.45 - 2.60 (m, 2 H), 1.59 (s, 3 H).

Example S43. Synthesis of Compound 26.

[0262] Compound **26** was synthesized by General Procedure C using 2-(2-bromoethyl)thiophene as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 464.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.48 (m, 2 H), 7.15-7.26 (m, 3 H), 6.85 – 6.95 (m, 2 H), 6.39 – 6.95 (m, 2 H),

5.90 – 6.20 (m, 1 H), 5.15 – 5.25 (m, 1 H), 3.72 – 3.96 (m, 2 H), 3.47 – 3.54 (m, 1 H), 3.32 - 3.42 (m, 3 H), 3.10 - 3.20 (m, 2 H), 2.42 - 2.56 (m, 2 H), 1.49 (s, 3 H).

Example S44. Synthesis of Intermediate Compound 1-(4-(difluoromethoxy)benzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione.



[0263] Step 1: Synthesis of (9*H*-fluoren-9-yl)methyl 6-methyl-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate. A solution of (9*H*-fluoren-9-yl)methyl 8-(4-methoxybenzyl)-6-methyl-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate (1.0 g, 26.63 mmol) in TFA (10 mL) was stirred at 130 °C for 2 h in microwave. After complete consumption of the starting material (monitored by TLC), the reaction mixture was concentrated under vacuum and the crude product was extracted with ethylacetate (100 ml) and saturated solution of sodium bicarbonate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum, and purified by column chromatography (Silica 100-200 mesh; 5% MeOH in DCM) to afford (9*H*-fluoren-9-yl)methyl 6-methyl-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate (300 mg, 42 % yield) as a sticky solid. MS (ESI) m/z $[M+H]^+$: 406.

[0264] Step 2: Synthesis of 6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione. To a solution of (9*H*-fluoren-9-yl)methyl 6-methyl-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate (300 mg, 0.74 mmol) in CH_2Cl_2 (5 mL) was added diethylamine (6 mL). The reaction mixture was stirred at room temperature for 3 h. After complete consumption of the starting material (monitored by TLC), the reaction mixture was concentrated and the crude product was purified by column chromatography (Silica 100-200mesh; 10% MeOH in DCM) to afford 6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (120 mg, 92% yield) as a white solid. MS (ESI) m/z $[M+H]^+$: 184.

[0265] Step 3: Synthesis of 1-(4-(difluoromethoxy)benzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione. To a solution of 6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6H)-dione (0.700 g, 3.820 mmol) in DMF (8.0 mL) was added

K_2CO_3 (1.58 g, 11.46 mmol) at room temperature and stirred for 10 min. To the resulting reaction mixture was added 1-(bromomethyl)-4-(difluoromethoxy)benzene (1.086 g, 4.584 mmol) and the reaction mixture was heated at 80 °C for 6 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was cooled to room temperature, quenched with water (50 mL) and extracted with EtOAc (50 mL × 2). The combined organic layers were washed with saturated brine solution (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (Silica 100-200 mesh; 5% MeOH in DCM) to afford 1-(4-(difluoromethoxy)benzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (0.550 g, 43.0% yield) as an off-white solid. MS (ESI) m/z $[M+H]^+$: 340.34.

Example S45. General Procedure D for Synthesis of Final Compounds.

[0266] To a solution of 1-(4-(difluoromethoxy)benzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (0.100 g,0.2949 mmol) in DMF (2 mL) was added NaH (0.021 g, 0.8847 mmol) at 0 °C followed by the appropriate alkyl halide (2 eq.) and the reaction mixture was allowed to warm to room temperature and stirred for 5 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was slowly quenched with saturated solution of aq. $NaHCO_3$ (2 mL) and extracted with EtOAc (10 mL × 2). The combined organic layers were washed with H_2O (5 mL) followed by saturated brine solution (5 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by Combiflash column chromatography (5% MeOH in DCM) to afford the final product.

Example S46. Synthesis of Compound 37.

[0267] Compound **37** was synthesized by General Procedure D using 4-bromo-1,1,1-trifluorobutane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 354.2. ^1H NMR (400 MHz, $CDCl_3$): δ 1.41 (d, J = 7.13 Hz, 3 H), 1.71 - 1.86 (m, 2 H), 2.01 - 2.15 (m, 2 H), 2.26 - 2.35 (m, 1 H), 2.60 - 2.67 (m, 1 H), 2.89 - 3.01 (m, 1 H), 3.07 - 3.15 (m, 1 H), 3.21 - 3.34 (m, 2 H), 3.46 - 3.65 (m, 2 H), 3.81 - 3.95 (m, 2 H), 4.35 - 4.41 (m, 1 H), 5.20 - 5.29 (m, 1 H), 6.53 (t, J = 72.0 Hz, 1 H), 7.08 - 7.16 (m, 2 H), 7.30 - 7.36 (m, 2 H).

Example S47. Synthesis of Compound 38.

[0268] Compound **38** was synthesized by General Procedure D using (2-bromoethyl)cyclopentane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 436.2. ^1H NMR (400 MHz, $CDCl_3$) δ 1.01 - 1.15 (m, 2 H), 1.41 (d, J = 7.13 Hz, 3 H), 1.45 - 1.62 (m, 8 H), 1.66 - 1.80 (m, 2 H), 2.23 - 2.34 (m, 1 H), 2.58 - 2.72 (m, 1 H), 2.89 - 2.98 (m, 1 H), 3.04 - 3.18 (m, 2 H), 3.23 - 3.33 (m, 1 H), 3.43 - 3.54 (m, 1 H), 3.55 - 3.65 (m, 1 H), 3.78 - 3.93 (m, 1 H), 4.31 - 4.39 (m, 1

H), 5.15 - 5.26 (m, 1 H), 6.53 (t, J = 72.0 Hz, 1 H), 7.13 (d, J = 8.50 Hz, 2 H), 7.34 (d, J = 8.50 Hz, 2 H).

Example S48. Synthesis of Compound 39.

[0269] Compound **39** was synthesized by General Procedure D using 4-bromobut-1-ene as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 394.2. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (d, J = 7.13 Hz, 3 H), 2.23 - 2.35 (m, 3 H), 2.60 - 2.71 (m, 1 H), 2.92 - 3.01 (m, 1 H), 3.06 - 3.14 (m, 1 H), 3.22 - 3.46 (m, 3 H), 3.53 - 3.64 (m, 1 H), 3.79 - 3.93 (m, 2 H), 4.28 - 4.38 (m, 1 H), 4.91 - 5.00 (m, 2 H), 5.16 - 5.26 (m, 1 H), 5.64 - 5.76 (m, 1 H), 6.52 (t, J = 72.0 Hz, 1 H), 7.10 - 7.16 (m, 2 H), 7.30 - 7.36 (m, 2 H).

Example S49. Synthesis of Compound 40.

[0270] Compound **40** was synthesized by General Procedure D using (2-bromoethyl)cyclobutene as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 422.25 ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (d, J = 7.13 Hz, 3 H), 1.56 - 1.65 (m, 4 H), 1.73 - 1.92 (m, 2 H), 1.95 - 2.07 (m, 2 H), 2.14 - 2.25 (m, 1 H), 2.26 - 2.35 (m, 1 H), 2.59 - 2.72 (m, 1 H), 2.91 - 2.99 (m, 1 H), 3.04 - 3.14 (m, 2 H), 3.23 - 3.43 (m, 2 H), 3.53 - 3.63 (m, 1 H), 3.87 (q, J = 13.38 Hz, 2 H), 4.29 - 4.39 (m, 1 H), 5.17 - 5.24 (m, 1 H), 6.53 (t, J = 72.0 Hz, 1 H), 7.13 (d, J = 8.63 Hz, 2 H), 7.30 - 7.37 (m, 2 H).

Example S50. Synthesis of Compound 41.

[0271] Compound **41** was synthesized by General Procedure D using 1-bromobutane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 396.05. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86 (t, J = 7.34 Hz, 3 H), 1.14 - 1.24 (m, 2 H), 1.24 - 1.30 (m, 2 H), 1.38 - 1.50 (m, 2 H), 1.98 - 2.10 (m, 1 H), 2.53 - 2.61 (m, 2 H), 2.64 - 2.77 (m, 2 H), 3.07 - 3.25 (m, 3 H), 3.32 - 3.41 (m, 1 H), 3.62 - 3.73 (m, 1 H), 3.87 - 3.93 (m, 2 H), 4.49 - 4.58 (m, 1 H), 4.84 - 4.94 (m, 1 H), 7.15 (d, J = 8.56 Hz, 2 H), 7.22 (t, J = 72.0 Hz, 1 H), 7.43 (d, J = 8.56 Hz, 1 H).

Example S51. Synthesis of Compound 52.

[0272] Compound **52** was synthesized by General Procedure D using 2-trifluromethyl-1-bromoethane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 420.16. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.31 - 7.38 (m, 2 H), 7.11 - 7.16 (m, 2 H), 6.31 - 6.73 (m, 1 H), 5.26 (q, J = 7.21 Hz, 1 H), 4.23 - 4.44 (m, 2 H), 3.98 - 4.13 (m, 1 H), 3.80 - 3.93 (m, 3 H), 3.59 (t, J = 11.07 Hz, 1 H), 3.10 (dd, J = 11.51, 3.75 Hz, 1 H), 2.90 – 2.99 (m, 1 H), 2.62 - 2.72 (m, 1 H), 2.32 (dd, J = 4.38, 2.38 Hz, 1 H), 2.28 (dd, J = 4.31, 2.31 Hz, 1 H), 1.48 (d, J = 7.25 Hz, 1 H), 1.41 (d, J = 7.13 Hz, 3 H).

Example S52. General Procedure E for the Synthesis of Final Compounds.

[0273] To a solution of 6-methyl-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (0.300 g, 1.184 mmol) in DMF (6 mL) stirred in a flask immersed

in an ice/water bath was added cesium carbonate (0.771 g, 2.368 mmol, 2 eq,) followed by the appropriate alkyl halide (1.1 eq.). The flask was removed from the bath and stirred until TLC indicated complete consumption of starting material. The reaction mixture was poured in ice-cold water (70 mL) and aqueous layer was extracted with EtOAc (100 mL). The organic layer was washed with ice cold brine (50 mL × 3), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by preparative HPLC to afford to give the final compound.

Example S53. Synthesis of Compound 42.

[0274] Compound **42** was synthesized by General Procedure E using 4-(bromomethyl)-2-chloro-1-(trifluoromethyl)benzene as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 362.2. ^1H NMR (400 MHz, DMSO-d_6) δ 0.75 - 0.89 (m, 3 H), 0.82 - 0.87 (m, 3 H), 0.96 - 1.13 (m, 1 H), 1.23 - 1.31 (m, 4 H), 1.64 - 1.75 (m, 1 H), 2.06 - 2.09 (m, 1 H), 2.55 - 2.62 (m, 1 H), 2.65 - 2.76 (m, 1 H), 3.05 - 3.15 (m, 1 H), 3.15 - 3.26 (m, 3 H), 3.64 - 3.74 (m, 1 H), 3.84 - 3.95 (m, 2 H), 4.52-4.60 (m, 1 H), 4.86 - 4.94 (m, 1 H), 7.17 (t, J = 8.76 Hz, 2 H), 7.41 (dd, J = 8.19, 5.82 Hz, 2 H).

Example S54. Synthesis of Compound 43.

[0275] Compound **43** was synthesized by General Procedure E using 4-(bromomethyl)-2-chloro-1-(trifluoromethyl)benzene as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 446.2. ^1H NMR (400 MHz, DMSO-d_6) 0.72-0.80 (m, 3 H), 0.80 - 0.87 (m, 3 H), 0.96 - 1.10 (m, 1 H),1.21 - 1.27 (m, 1 H), 1.28 - 1.34 (m, 3 H), 1.62 - 1.79 (m, 1 H), 2.00 - 2.13 (m, 1 H), 2.53 - 2.65 (m, 1 H), 2.66 - 2.76 (m, 1 H), 3.00 - 3.10 (m, 1 H), 3.17 - 3.29 (m, 3 H), 3.62 - 3.72 (m, 1 H), 4.00 - 4.08 (m, 2 H), 4.55 - 4.65 (m, 1 H), 4.85 - 4.95 (m, 1 H), 7.52 - 7.60 (m, 1 H), 7.73 (s, 1 H), 7.80 - 7.88 (m, 1 H).

Example S55. Synthesis of Compound 44.

[0276] To a solution of 6-methyl-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (0.420 g, 1.657 mmol) and 1*H*-indole-3-carbaldehyde (0.264 g, 1.823 mmol) in DCE (15 mL) was added acetic acid (1 mL, 1.657 mmol) and heated the reaction mixture at 80 °C for 1 h. To the resulting reaction mixture was added portion wise $NaBH_4$ (0.188 g, 4.973 mmol) and the reaction mixture was heated at 80 °C and stirred for 4 h. When TLC analysis (5% MeOH in DCM) indicated complete consumption of the starting material the reaction mixture was diluted with water (40 mL) and aqueous layer was extracted with DCM (100 mL). The organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200 mesh; 5% MeOH in DCM) followed by washing with water (30 mL) and dried under reduced pressure to afford Compound **44** (0.250 g, 39% yield) as an off white solid. MS (ESI) m/z $[M+H]^+$: 383.4. ^1H NMR (400 MHz, DMSO-d_6) δ 0.70 (t, J = 7.09

Hz, 3 H), 0.75 - 0.82 (m, 3 H), 0.91 - 1.11 (m, 1 H), 1.22 - 1.31 (m, 3 H), 1.57 - 1.72 (m, 1 H), 1.97 - 2.07 (m, 1 H), 2.55 - 2.70 (m, 2 H), 2.83 (dt, *J* = 10.91, 2.74 Hz, 1 H), 2.95 - 3.07 (m, 1 H), 3.10 - 3.26 (m, 3 H), 3.54 - 3.69 (m, 1 H), 3.96 - 4.04 (m, 1 H), 4.06 - 4.15 (m, 1 H), 4.54 - 4.64 (m, 1 H), 4.84 - 4.95 (m, 1 H), 6.94 - 7.02 (m, 1 H), 7.04 - 7.13 (m, 1 H), 7.29 - 7.40 (m, 2 H), 7.65 (d, *J* = 7.95 Hz, 1 H), 10.95 (s, 1 H).

Example S56. Synthesis of Intermediate Compound 1-(4-fluorobenzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*) dione.

[0277] To a solution of 6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (250 mg, 1.40 mmol) in DMF (3 mL) was added potassium carbonate (580 mg, 4.20 mmol) followed by 4-fluorobenzylbromide (0.320 g, 1.70 mmol) and stirred at 80°C temperature for 3 h. After completion, the reaction mixture was monitored by TLC (5% MeOH in DCM). The reaction mixture was poured in ice-cold water (50 mL) and aqueous layer was extracted with EtOAc (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200mesh; 5% MeOH in DCM) to afford 1-(4-fluorobenzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*) dione (160 mg, 70% yield) as a white solid. MS (ESI) m/z $[M+H]^+$: 292.

Example S57. Synthesis of Compound 45.

[0278] To a solution of 1-(4-fluorobenzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*) dione (80 mg, 0.2739 mmol) in DMF (3 mL) under an ice cold bath at 0 °C was added NaH (20 mg, 0.2739 mmol) and stirred for 20 min then added (2-bromoethyl)cyclobutane (67 mg, 0.41 mmol) after 3 h. After complete consumption of starting material (monitored by TLC), the reaction mixture was quenched with ice cold water and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200mesh; 5% MeOH in DCM) to afford Compound **45** (8-(2-cyclobutylethyl)-1-(4-fluorobenzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione) (13 mg, 16% yield) as a gummy liquid. MS (ESI) m/z $[M+H]^+$: 374. ^1H NMR (400 MHz, CD_3Cl_3): δ 7.30 - 7.40 (m, 2H), 7.00 - 7.10 (m, 2H), 5.15 – 5.25 (m, 1H), 4.25 - 4.35 (m, 1H), 3.80 – 3.95 (m, 2H), 3.55 – 3.65 (m, 1H), 3.25 – 3.45 (m, 2H), 3.05 – 3.20 (m, 2H), 2.90 – 3.0 (m, 1H), 2.60 – 2.70 (m, 1H), 2.15 – 2.40 (m, 2H), 1.75 – 2.10 (m, 4H), 1.55 – 1.65 (m, 4H), 1.20 – 1.30 (m, 3H).

Example S58. Synthesis of Compound 46.

[0279] To a solution of 1-(4-fluorobenzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*) dione (80 mg, 0.2739 mmol) in DMF (3 mL) under an ice cold bath at 0

°C was added NaH (20 mg, 0.2739 mmol) and stirred for 20 min, then was added (2-bromoethyl)cyclopentane (72 mg, 0.41 mmol) after 3 hours, completion of starting material monitored by TLC, the reaction mixture was quenched with ice cold water and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200mesh; 5% MeOH in DCM) to afford Compound **46** (8-(2-cyclopentylethyl)-1-(4-fluorobenzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione) as a gummy liquid.

Example S59. Synthesis of Intermediate Compound 6-(fluoromethyl)-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione Hydrochloride salt.

[0280] Step 1: Synthesis of *N*-(2,2-diethoxyethyl)-2-methylbutan-1-amine. To stirred neat 2,2-diethoxyethan-1-amine (20.0 g, 0.137 mmol) was added 2-methylbutanal (11.60 g, 0.137 mmol) at room temperature and the reaction mixture was heated to 100 °C for 3 h. To the resulting reaction mixture was slowly added ethanol (200 mL) followed by $NaBH_4$ (15.40 g, 0.413 mmol) at room temperature and the reaction mixture was stirred for 16 h. After complete consumption of starting material (monitored by TLC),.the reaction mixture was cooled to room temperature and slowly quenched with a saturated solution of NH_4Cl (100 mL). The aq. layer was extracted with EtOAc (200 mL x 2). The combined organic layer was washed with brine (400 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to get crude compound. The crude obtained was purified by column chromatography (silica 100-200 mesh; 10% MeOH in DCM) to obtain *N*-(2,2-diethoxyethyl)-2-methylbutan-1-amine (25.8 g, 88% yield) colorless liquid. MS (ESI) m/z $[M+H]^+$: 204.3. ^1H NMR (400 MHz, DMSO-d6) δ 0.80 - 0.89 (m, 6 H) 1.11 (t, J=6.98 Hz, 6H) 1.35 - 1.48 (m, 2 H) 2.28-2.32 (m, 1 H) 2.41-2.45 (m, 1 H) 2.55 (d, J=5.49 Hz, 2 H) 3.42 - 3.52 (m, 2 H) 3.57 - 3.65 (m, 2 H) 4.49 (t, J=5.49 Hz, 1 H).

[0281] Step 2: (9*H*-fluoren-9-yl)methyl (1-((2,2-diethoxyethyl)(2-methylbutyl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate. To a stirred solution of (((9*H*-fluoren-9-yl)methoxy)carbonyl)serine (15.0 g, 45.81 mmol) in dry DMF (150 mL) maintained at 0 °C was added HATU (26.0 g, 68.80 mmol), DIPEA (23.92 mL, 137.61 mmol) followed by *N*-(2,2-diethoxyethyl)-2-methylbutan-1-amine (12.10 g, 59.63 mmol). The reaction mixture was stirred at room temperature for 4 h. After complete consumption of starting material, the reaction mixture was quenched with ice cold water (500 mL) and the aqueous layer was extracted with EtOAc (250 mL x 2). The combined organic layer was washed with cold H_2O (200 mL) followed by brine (200 mL), dried over Na_2SO_4 and concentrated under reduced pressure to provide the crude product. The crude material was purified by column chromatography (Silica 100-200 mesh; 80% EtOAc in hexanes) to afford (9*H*-fluoren-9-yl)methyl (1-((2,2-

diethoxyethyl)(2-methylbutyl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate (21.0 g, 89.43% yield) as yellow sticky solid. MS (ESI) m/z $[M+Na]^+$: 535.35.

[0282] Step 3: Synthesis of 2-amino-*N*-(2,2-diethoxyethyl)-3-hydroxy-*N*-(2-methylbutyl)propenamide. To a stirred solution of (9*H*-fluoren-9-yl)methyl (1-((2,2-diethoxyethyl)(2-methylbutyl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate (21.0 g, 41.01 mmol) in dry DCM (110 mL) maintained at 0 °C was added diethylamine (58 mL, 2.80 volume) and reaction mixture was stirred at room temperature for 3 h. After complete consumption of starting material (monitored by TLC), the reaction mixture was concentrated under reduced pressure to get crude product. The crude obtained was purified by column chromatography (Silica 100-200 mesh; 5% MeOH in DCM) to afford 2-amino-*N*-(2,2-diethoxyethyl)-3-hydroxy-*N*-(2-methylbutyl)propenamide (9.50 g, 80 % yield) as yellow sticky solid. MS (ESI) m/z $[M+H]^+$: 291.4.

[0283] Step 4: Synthesis of (9*H*-fluoren-9-yl)methyl (3-((1-((2,2-diethoxyethyl)(2-methylbutyl)amino)-3-hydroxy-1-oxopropan-2-yl)amino)-3-oxopropyl)carbamate. To a stirred solution of 3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid (9.50 g, 30.54 mmol) in dry DMF (95 mL) maintained at 0 °C was added HATU (17.40 g, 45.81 mmol), DIPEA (16.0 mL, 91.62 mmol) followed by 2-amino-*N*-(2,2-diethoxyethyl)-3-hydroxy-*N*-(2-methylbutyl)propanamide (13.20 g, 45.81 mmol) at room temperature and the reaction mixture was stirred for 16 h. After completion, the reaction mixture was quenched with ice cold water (200 mL) and the aqueous layer was extracted with EtOAc (200 mL x 2). The organic layer was washed with cold H_2O (500 mL) followed by saturated brine (200 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by column chromatography (Silica 100-200 mesh; 80% EtOAc in Hexanes) to afford (9*H*-fluoren-9-yl)methyl (3-((1-((2,2-diethoxyethyl)(2-methylbutyl)amino)-3-hydroxy-1-oxopropan-2-yl)amino)-3-oxopropyl)carbamate (8.0 g, 31.0 % yield) as a viscous yellow oil. MS (ESI) m/z $[M-H]^-$: 582.2.

[0284] Step 5: Synthesis of (9*H*-fluoren-9-yl)methyl 6-(hydroxymethyl)-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate. A stirred solution of (9*H*-fluoren-9-yl)methyl (3-((1-((2,2-diethoxyethyl)(2-methylbutyl)amino)-3-hydroxy-1-oxopropan-2-yl)amino)-3-oxopropyl)carbamate (8.0 g, 13.77 mmol) in formic acid (48.0 mL, 6.0 volume) at room temperature and reaction mixture was stirred for 16 h. After completion, the reaction mixture was concentrated under reduced pressure to afford (9*H*-fluoren-9-yl)methyl 6-(hydroxymethyl)-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate (6.0 g, crude) as brown semi-solid. The crude compound was used as such for next reaction without further purification. MS (ESI) m/z $[M+H]^+$: 492.2.

[0285] Step 6: Synthesis of 6-(hydroxymethyl)-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione. To a solution of (9*H*-fluoren-9-yl)methyl 6-(hydroxymethyl)-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate (6.0 g, 12.20 mmol) in CH_2Cl_2 (36.0 mL) was added diethylamine (18.0 mL) at 0 °C and the reaction mixture was stirred at room temperature for 3 h. After complete consumption of the starting material (monitored by TLC), the reaction mixture was concentrated under reduced pressure to obtain the crude compound. The crude material was purified by column chromatography (Silica 100-200 mesh; 5% MeOH in DCM) to afford 6-(hydroxymethyl)-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (3.0 g, 93.75% yield) as a viscous colorless oil. MS (ESI) m/z $[M+H]^+$: 270.20.

[0286] Step 7: Synthesis of *tert*-butyl 6-(hydroxymethyl)-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate. To a solution of 6-(hydroxymethyl)-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (3.0 g, 11.15 mmol) in CH_2Cl_2 (60 mL) was added triethylamine (4.5 mL, 33.45 mmol) followed by Boc anhydride (3.78 mL, 16.72 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 16 h. After complete consumption of the starting material (monitored by TLC), the reaction mixture was slowly quenched with ice cold water (30 mL) and extracted with DCM (40 mL). The organic layer was washed with brine (30 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by column chromatography (Silica 100-200 mesh; 10% MeOH in DCM) to afford *tert*-butyl 6-(hydroxymethyl)-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate (8.0 g, 31.0 % yield) as a viscous yellow oil. MS (ESI) m/z $[M+H]^+$: 370.25.

[0287] Step 8: Synthesis of *tert*-butyl 6-(fluoromethyl)-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate. To a solution of *tert*-butyl 6-(hydroxymethyl)-8-(2-methylbutyl)-4,7-dioxohexahydro-2H-pyrazino[1,2-a]pyrimidine-1(6H)-carboxylate (1.50 g, 4.065 mmol) in DCM (30 mL) was added DAST (1.97 g, 12.19 mmol) at -78 °C and stirred for 15 min. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. After completion of the reaction (monitored by TLC), the reaction mixture was quenched with saturated $NaHCO_3$ solution (15 mL) and the aqueous layer was extracted with EtOAc (100 mL x 2). The combined organic layer was washed with saturated brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure to obtain crude compound. The crude material was purified by column chromatography (Silica 100-200 mesh; 5% MeOH in DCM) to afford *tert*-butyl 6-(fluoromethyl)-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate (0.800 g, 72.0 % yield) as a colorless viscous oil. MS (ESI) m/z $[M+H]^+$: 372.2.

[0288] Step 9: Synthesis of 6-(fluoromethyl)-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione Hydrochloride salt. To a stirred solution of tert-butyl 6-(fluoromethyl)-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate (1.0 g, 2.695 mmol) in 1,4-dioxane (5 mL) was added 4 M HCl in dioxane (5 mL) at 0 °C and the reaction mixture was stirred at room temperature for 3 h. After complete consumption of the starting material (monitored by TLC), the reaction mixture was quenched with saturated solution of sodium bicarbonate (10 mL) and extracted with ethyl acetate (20 mL x 3). The combined organic layer was washed with saturated brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure to afford 6-(fluoromethyl)-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione hydrochloride salt (0.630 g, crude) as a brown sticky oil. MS (ESI) m/z $[M+H]^+$ free base: 271.00.

Example S60. Synthesis of Compound 47.

[0289] To a solution of 6-(fluoromethyl)-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione hydrochloride salt (0.150 g, 0.550 mmol) in DMF (1.5 mL) was added K_2CO_3 (0.381 g, 2.760 mmol) followed by 1-(bromomethyl)-4-(difluoromethoxy)benzene (0.261 g, 1.100 mmol) and the reaction mixture was stirred at room temperature for 16 h. After completion (monitored by TLC), the reaction mixture was slowly quenched with ice cold water (6 mL) and extracted with EtOAc (20 mL×3). The combined organic layer was washed with saturated brine solution (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure to get crude compound. The crude compound was purified by Prep HPLC to afford Compound **47** (1-(4-(difluoromethoxy)benzyl)-6-(fluoromethyl)-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione) (0.040 g, 17.0% yield) as a white solid. MS (ESI) m/z $[M+H]^+$: 428.10. ^1H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, J =8.01, 2 H), 7.11 (d, J =8.01, 2 H), 6.32 – 6.69 (m, 1 H), 5.14-5.25 (m, 2 H), 4.60 – 4.76 (m, 2 H), 3.84 – 3.97 (m, 2 H), 3.35 – 3.45 (m, 2 H), 3.12 – 3.40 (m, 4 H), 2.85 – 3.05 (m, 1 H), 2.65 – 2.75 (m, 1 H), 2.29 – 2.34 (m, 1 H), 1.65 – 1.75 (m, 1H), 1.30 – 1.40 (m, 1 H), 1.05 -1.18 (m, 1 H), 0.80 – 0.90 (m, 6 H).

Example S61. Synthesis of Compound 48.

[0290] To a solution of 6-(fluoromethyl)-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione hydrochloride salt (0.340 g, 1.253 mmol) in DMF (3.4 mL) was added Cs_2CO_3 (0.814 g, 2.506 mmol) followed by 1-(bromomethyl)-4-(trifluoromethyl)benzene (0.598 g, 2.506 mmol), and reaction mixture was stirred at room temperature for 16 h. After completion (monitored by TLC), the reaction mixture was slowly quenched with ice cold water (6 mL) and extracted with EtOAc (20 mL×3). The combined organic layer was washed with saturated brine solution (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure to

get crude compound. The crude compound was purified by prep HPLC to afford Compound **48** (6-(fluoromethyl)-8-(2-methylbutyl)-1-(4-(trifluoromethyl)benzyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione) (0.045 g, 8.0% yield) as a white solid. MS (ESI) m/z $[M+H]^+$: 430.10. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, J =8.01, 2 H), 7.11 (d, J =8.01, 2 H), 5.14-5.25 (m, 2 H), 4.60 – 4.76 (m, 2 H), 3.84 – 3.97 (m, 2 H), 3.35 – 3.45 (m, 2 H), 3.12 – 3.40 (m, 4 H), 2.85 – 3.05 (m, 1 H), 2.65 – 2.75 (m, 1 H), 2.29 – 2.34 (m, 1 H), 1.65 – 1.75 (m, 1H), 1.30 – 1.40 (m, 1 H), 1.05 -1.18 (m, 1 H), 0.80 – 0.90 (m, 6 H).

Example S62. Synthesis of Intermediate Compound methyl 2-(1-(4-(difluoromethoxy)benzyl)-8-(2-methylbutyl)-4,7-dioxooctahydro-2*H*-pyrazino[1,2-*a*]pyrimidin-6-yl)acetate.

[0291] Step 1: Synthesis of methyl 3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-oxobutanoate. To a solution 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-methoxy-4-oxobutanoic acid (1.90 g, 9.475 mmol) stirred at 0 °C in dry DMF (30 mL) was added HATU (3.60 g, 1.137 mmol) followed by DIPEA (2.70 mL, 1.895 mmol), and the reaction mixture was stirred at same temperature for 10 min. To the resulting reaction mixture was added *N*-(2,2-diethoxyethyl)-2-methylbutan-1-amine (3.50 g, 9.475 mmol), then the mixture was allowed to warm to room temperature and stirred for 6 h. After complete consumption of the starting material (monitored by TLC), the reaction mixture was quenched with ice cold water (100 mL) and the aqueous layer was extracted with EtOAc (50 mL x 2). The combined organic layer was washed with cold H_2O (50 mL) followed by brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure to get crude product. The crude material was purified by CombiFlash column chromatography using 50% EtOAc in *n*-hexanes to afford methyl 3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-oxobutanoate (4.30 g, 83.0% yield) as a white solid. MS (ESI) m/z $[M+H-EtOH]^+$: 509.2.

[0292] Step 2: Synthesis of methyl 3-amino-4-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-oxobutanoate. To a solution of methyl 3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-oxobutanoate (1.36 g, 2.451 mmol) in CH_2Cl_2 (27.0 mL) was added diethylamine (1.53 mL, 14.71 mmol) at room temperature and the reaction mixture was stirred for 3 h. After complete consumption of the starting material (monitored by TLC), the reaction mixture was concentrated under reduced pressure to obtained crude compound. The crude compound was purified by CombiFlash column chromatography using 5% MeOH in DCM to afford methyl 3-amino-4-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-oxobutanoate (0.700 g, 86% yield) as yellow viscous liquid. MS (ESI) m/z $[M+H-EtOH]^+$**:** 287.68.

[0293] Step 3: Synthesis of methyl 3-(3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-oxobutanoate. To a stirred solution of 3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid (0.490 g, 1.594 mmol) in dry DMF (10 mL) maintained at 0°C was added HATU (0.720 g, 1.913 mmol), DIPEA (0.555 mL, 3.188 mmol) followed by the addition of methyl 3-amino-4-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-oxobutanoate (0.530 g, 1.594 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 6 h. After completion, the reaction mixture was quenched with ice cold water (20 mL) and the aqueous layer was extracted with EtOAc (20 mL x 2). The organic layer was washed with cold H_2O (10 mL) followed by saturated brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by Combiflash column chromatography using 5% MeOH in DCM to afford methyl 3-(3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-oxobutanoate (0.630 g, 70 % yield) as an off-white solid. MS (ESI) m/z $[M+H-EtOH]^+$: 580.20.

[0294] Step 4: Synthesis of (9*H*-fluoren-9-yl)methyl 6-(2-methoxy-2-oxoethyl)-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate. To a stirred solution of methyl 3-(3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-oxobutanoate (0.300 g, 0.4794 mmol) was added formic acid (1.5 mL) at room temperature and the reaction mixture was stirred for 16 h. After completion, the reaction mixture was concentrated and the crude obtained was purified by column chromatography (Silica 100-200 mesh; 0-5% MeOH in DCM) to afford (9*H*-fluoren-9-yl)methyl 6-(2-methoxy-2-oxoethyl)-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate (0.200 g, 80% yield) as a yellow solid. MS (ESI) m/z $[M+H]^+$: 534.67.

[0295] Step 5: Synthesis of methyl 2-(8-(2-methylbutyl)-4,7-dioxooctahydro-2*H*-pyrazino[1,2-*a*]pyrimidin-6-yl)acetate. To a solution of (9*H*-fluoren-9-yl)methyl 6-(2-methoxy-2-oxoethyl)-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate (0.240 g, 0.4499 mmol) in CH_2Cl_2 (0.5 mL) was added diethylamine (0.280 mL) and the reaction mixture was stirred at room temperature for 3 h. After complete consumption of the starting material (monitored by TLC), the reaction mixture was concentrated and the crude material was purified by Combiflash column chromatography using 0-5% MeOH in DCM to afford methyl 2-(8-(2-methylbutyl)-4,7-dioxooctahydro-2*H*-pyrazino[1,2-*a*]pyrimidin-6-yl)acetate (0.130 g, 93% yield) as white solid. MS (ESI) m/z $[M-H]^+$: 310.4.

[0296] **Step 6: Synthesis of methyl 2-(1-(4-(difluoromethoxy)benzyl)-8-(2-methylbutyl)-4,7-dioxooctahydro-2*H*-pyrazino[1,2-*a*]pyrimidin-6-yl)acetate**. To a solution of methyl 2-(8-(2-methylbutyl)-4,7-dioxooctahydro-2*H*-pyrazino[1,2-*a*]pyrimidin-6-yl)acetate (3.08 g, 9.890 mmol) in DMF (30 mL) was added K_2CO_3 (4.10 g, 29.66 mmol) at room temperature, and reaction mixture stirred at 80 °C for 15 min. To the resulting reaction mixture was added 1-(bromomethyl)-4-(difluoromethoxy)benzene (3.48 g, 14.36 mmol) and the stirred mixture was heated to 80 °C for 2 h. After completion, the reaction mixture was quenched with ice cold water (200 mL) and the aqueous layer was extracted with EtOAc (200 mL x 2). The organic layer was washed with cold H_2O (200 mL) followed by saturated brine (150 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound obtained was purified by Combiflash column chromatography (5% MeOH in DCM) to afford methyl 2-(1-(4-(difluoromethoxy)benzyl)-8-(2-methylbutyl)-4,7-dioxooctahydro-2*H*-pyrazino[1,2-*a*]pyrimidin-6-yl)acetate (2.20 g, 48 % yield) as a yellow solid. MS (ESI) m/z $[M-CH_3]^+$: 454.10.

Example S63. Synthesis of Compound 49.

[0297] To a solution of methyl 2-(1-(4-(difluoromethoxy)benzyl)-8-(2-methylbutyl)-4,7-dioxooctahydro-2*H*-pyrazino[1,2-*a*]pyrimidin-6-yl)acetate (2.20 g, 4.705 mmol) in THF (22.0 mL) was added NaOH (0.560 g, 14.11 mmol) followed by water (4 mL) and the reaction mixture was stirred at room temperature for 3 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was concentrated under reduced pressure. The crude residue was dissolved in water (10 mL), slowly acidified with 6N HCl (10 mL) and stirred for 5 min. The obtained solid precipitate was filtered through a Buchner funnel and dried under reduced pressure to afford Compound **49** (2-(1-(4-(difluoromethoxy)benzyl)-8-(2-methylbutyl)-4,7-dioxooctahydro-2*H*-pyrazino[1,2-*a*]pyrimidin-6-yl)acetic acid) (0.85 g, 40% yield) as a white solid. MS (ESI) m/z $[M+H]^+$: 454.10. ^1H NMR (400 MHz, $CDCl_3$) δ 7.28 – 7.38 (m, 2 H), 7.11 (d, J = 7.99 Hz, 2 H), 6.33 – 6.71 (m, 1 H), 5.36 – 5.40 (m, 1 H), 4.70 – 4.80 (m, 1 H), 4.65 – 4.75 (m, 1 H), 3.80 – 4.00 (m, 2 H), 3.55 – 3.65 (m, 1 H), 3.35 – 3.45 (m, 1 H), 2.85 – 3.30 (m, 6 H), 2.70 – 2.80 (m, 1 H), 2.25 – 2.35 (m, 1 H), 1.65 – 1.76 (m, 1 H), 1.25 – 1.35 (m, 1H), 1.10 – 1.20 (m, 1H), 0.8 – 0.9 (m, 6 H).

Example S64. Synthesis of Compound 50.

[0298] To a solution of 2-(1-(4-(difluoromethoxy)benzyl)-8-(2-methylbutyl)-4,7-dioxooctahydro-2*H*-pyrazino[1,2-*a*]pyrimidin-6-yl)acetic acid (0.470 g, 1.036 mmol) in THF (5 mL) was added 1,1′-carbonyldiimidazole (0.500 g, 3.109 mmol) at room temperature and the reaction mixture was stirred for 15 min. To the resulting reaction mixture was added aq. NH_3 (10 mL) and reaction mixture was stirred at room temperature for 3 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was slowly quenched with ice cold

water (6 mL) and extracted with EtOAc (20 mL×3). The combined organic layer was washed with saturated brine solution (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure to provide the crude compound. The crude compound obtained was purified by Combiflash column chromatography using 5% MeOH in DCM followed by PREP HPLC to afford Compound **50** (2-(1-(4-(difluoromethoxy)benzyl)-8-(2-methylbutyl)-4,7-dioxooctahydro-2*H*-pyrazino[1,2-*a*]pyrimidin-6-yl)acetamide) (0.070 g, 15% yield) as a white solid. MS (ESI) m/z $[M+H]^+$: 453.20. ^1H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.40 (m, 2 H), 7.05-7.15 (m, 2 H), 6.39 – 6.70 (m, 1 H), 5.20 – 5.40 (m, 2 H), 4.75 – 4.85 (m, 1 H), 3.95 – 4.05 (m, 1 H), 3.75 – 3.85 (m, 1 H), 3.50 – 3.60 (m, 1 H), 3.30 - 3.40 (m, 1 H), 3.05 - 3.25 (m, 2 H), 2.85 - 2.95 (m, 2 H), 2.55 – 2.70 (m, 1 H), 2.25 – 2.35 (m, 1H), 1.70 – 1.80 (m, 2H), 1.30 – 1.40 (m, 2H), 1.05 – 1.20 (m, 2H), 0.75 – 0.90 (m, 6H).

Example S65. Synthesis of Intermediate Compound 1-(3-chloro-4-(trifluoromethyl)benzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione.

[0299] To a solution of 6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (500 mg, 2.732 mmol) in DMF (7 mL) was added potassium carbonate (1.13 g, 8.196 mmol) followed by 4-(bromomethyl)-2-chloro-1-(trifluoromethyl)benzene (0.894 g, 3.278 mmol) and stirred at 80 °C temperature for 12 h. After completion of the reaction, monitored by TLC (5% MeOH in DCM). The reaction mixture was poured in ice-cold water (50 mL) and the aqueous layer was extracted with EtOAc (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200mesh; 5% MeOH in DCM) to afford 1-(3-chloro-4-(trifluoromethyl)benzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (320 mg, 42% yield) as a white solid. MS (ESI) m/z $[M+H]^+$: 376.34.

Example S66. General Procedure F for the Synthesis of Final Compounds.

[0300] To a solution of 1-(3-chloro-4-(trifluoromethyl)benzyl)-6-methylhexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (150 mg, 0.400 mmol) in DMF (2 mL) at 0 °C was added Cs_2CO_3 (4 eq) and stirred for 20 min, then was added the appropriate alkyl halide (1.2 eq) at room temperature and the reaction mixture was heated at 80 °C and stirred for 12 h. After consumption of starting material (monitored by TLC), the reaction mixture was quenched with ice cold water and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude obtained was purified by column chromatography (Silica 100-200mesh; 5% MeOH in DCM) to give the final compounds.

Example S67. Synthesis of Compound 51.

[0301] Compound **51** was synthesized by General Procedure F using (2-bromoethyl)cyclobutane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 458.2. ^1H NMR (400 MHz,

$CDCl_3$) δ ppm 7.70 (d, *J* = 8.07 Hz, 1 H), 7.54 (s, 1 H), 7.33 (d, *J* = 8.68 Hz, 1 H), 4.34 (dd, *J* = 10.64, 3.55 Hz, 1 H), 3.87 - 3.99 (m, 2 H), 3.61 (t, *J* = 11.13 Hz, 1 H), 3.25 - 3.42 (m, 2 H), 3.08 - 3.19 (m, 2 H), 2.89 - 2.98 (m, 1 H), 2.64 – 2.74 (m, 1 H) 2.29 - 2.38 (m, 1 H) 2.17 - 2.27 (m, 1 H) 1.97 - 2.09 (m, 2 H) 1.72 - 1.92 (m, 3 H) 1.58 - 1.66 (m, 4 H) 1.55 (br. s, 3 H).

Example S68. Synthesis of Compound 54.

[0302] Compound **54** was synthesized by General Procedure F using (2-bromoethyl)cyclopentane as the alkyl halide. MS (ESI) m/z $[M+H]^+$: 472.15. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 7.69 (d, *J* = 8.11 Hz, 1 H) 7.52 - 7.56 (m, 1 H) 7.33 (d, *J* = 7.89 Hz, 1 H), 5.23 (q, *J* = 7.23 Hz, 1 H), 4.36 (dd, *J* = 10.52, 3.29 Hz, 1 H), 3.87 - 3.98 (m, 2 H), 3.63 (t, *J* = 11.07 Hz, 1 H), 3.46 - 3.56 (m, 1 H), 3.25 - 3.35 (m, 1 H), 3.12 - 3.23 (m, 2 H), 2.87 - 2.97 (m, 1 H), 2.60 – 2.70 (m, 1 H), 2.29 - 2.37 (m, 1 H), 1.66 - 1.82 (m, 2 H), 1.54 - 1.63 (m, 1 H), 1.51 (d, *J* =,2.63 Hz, 2 H), 1.42 (d, *J* = 7.23 Hz, 3 H), 1.26 (br. s, 2 H) 1.04 - 1.16 (m, 2 H) 0.80 - 0.92 (m, 2 H).

Example S69. Synthesis of Compound 53.

[0303] Step 1: Synthesis of (9*H*-fluoren-9-yl)methyl (1-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate. To a stirred solution of (((9*H*-fluoren-9-yl)methoxy)carbonyl)leucine (20.0 g, 56.58 mmol) in dry DMF (200 mL) was added HATU (21.50 g, 56.58 mmol) followed by DIPEA (10.62 mL, 61.10 mmol) at 0 °C and the reaction mixture was stirred at same temperature for 10 min. To the resulting reaction mixture was added *N*-(2,2-diethoxyethyl)-2-methylbutan-1-amine (11.48 g, 56.58 mmol) at room temperature and the reaction mixture was stirred for 3 h. After complete consumption of the starting material (monitored by TLC), the reaction mixture was quenched with ice cold water (100 mL) and the aqueous layer was extracted with EtOAc (50 mL x 4). The combined organic layers were washed with cold H_2O (50 mL x 2) followed by brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure to get crude product. The crude product was purified by CombiFlash column chromatography using 5% MeOH in DCM to afford (9*H*-fluoren-9-yl)methyl (1-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (14.5 g, 47.57 % yield) as a white solid. MS (ESI) m/z $[M+H]^+$: 539.04.

[0304] Step 2: Synthesis of 2-amino-*N*-(2,2-diethoxyethyl)-4-methyl-*N*-(2-methylbutyl)pentanamide. To a solution of (9*H*-fluoren-9-yl)methyl (1-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (8.50 g, 15.77 mmol) in CH_2Cl_2 (50 mL) was added diethylamine (16 mL, 157.7 mmol) at room temperature and the reaction mixture was stirred for 3 h. After complete consumption of the starting material (monitored by TLC), the reaction mixture was concentrated under reduced pressure to obtained crude compound. The crude compound was purified by CombiFlash column chromatography using

5% MeOH in DCM to afford 2-amino-*N*-(2,2-diethoxyethyl)-4-methyl-*N*-(2-methylbutyl)pentanamide (3.60 g, 72% yield) as a yellow viscous liquid. MS (ESI) m/z [M+H-EtOH]$^+$: 272.10.

[0305] Step 3: Synthesis of (9*H*-fluoren-9-yl)methyl (3-((1-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-3-oxopropyl)carbamate. To a stirred solution of 3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid (3.80 g, 12.28 mmol) in dry DMF (35 mL) maintained at 0°C was added HATU (6.48 g, 17.05 mmol) and DIPEA (4.90 mL, 28.42 mmol), followed by the addition of 2-amino-*N*-(2,2-diethoxyethyl)-4-methyl-*N*-(2-methylbutyl)pentanamide (3.60 g, 11.37 mmol). The reaction mixture was allowed to attain room temperature and stirred for 3 h. After completion, the reaction mixture was quenched with ice cold water (20 mL) and the aqueous layer was extracted with EtOAc (30 mL x 2). The organic layer was washed with cold H_2O (10 mL) followed by saturated brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by Combiflash column chromatography using 5% MeOH in DCM to afford (9*H*-fluoren-9-yl)methyl (3-((1-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-3-oxopropyl)carbamate (3.8 g, 55 % yield) as an off-white solid. MS (ESI) m/z [M+H-EtOH]$^+$: 565.30.

[0306] Step 4: Synthesis of (9*H*-fluoren-9-yl)methyl 6-isobutyl-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate. To a stirred solution of (9*H*-fluoren-9-yl)methyl (3-((1-((2,2-diethoxyethyl)(2-methylbutyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-3-oxopropyl)carbamate (3.80 g, 6.231 mmol) was added formic acid (20 mL) at room temperature and the reaction mixture was stirred for 16 h. After completion, the reaction mixture was concentrated under reduced pressure. The crude compound was purified by column chromatography (Silica 100-200 mesh; 0-5% MeOH in DCM) to afford (9*H*-fluoren-9-yl)methyl 6-isobutyl-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate (3.60 g, 94% yield) as a yellow solid. MS (ESI) m/z [M+H]$^+$: 518.23.

[0307] Step 5: Synthesis of 6-isobutyl-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione. To a solution of (9*H*-fluoren-9-yl)methyl 6-isobutyl-8-(2-methylbutyl)-4,7-dioxohexahydro-2*H*-pyrazino[1,2-*a*]pyrimidine-1(6*H*)-carboxylate (3.60 g, 6.954 mmol) in CH_2Cl_2 (36 mL) was added diethylamine (6.8 mL, 69.54 mmol) and the reaction mixture was stirred at room temperature for 16 h. After complete consumption of the starting material (monitored by TLC), the reaction mixture was concentrated under reduced pressure and the crude product was purified by Combiflash column chromatography using 10-50% ethyl acetate in *n*-hexane to afford 6-isobutyl-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (1.20 g, 60% yield) as a white solid. MS (ESI) m/z [M+H]$^+$: 296.10.

[0308] **Step 6: Synthesis of 1-(4-(difluoromethoxy)benzyl)-6-isobutyl-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione**. To a solution of 6-isobutyl-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione (0.170 g, 0.576 mmol) in DMF (5 mL) was added K_2CO_3 (0.159 g, 1.152 mmol) at 0 °C, and the reaction mixture was stirred for 10 min. To the resulting reaction mixture was added 1-(bromomethyl)-4-(difluoromethoxy)benzene (0.150 g, 0.632 mmol) at room temperature and stirred for 3 h. After completion, the reaction mixture was quenched with ice cold water (200 mL) and the aqueous layer was extracted with EtOAc (20 mL x 2). The organic layer was washed with cold H_2O (20 mL) followed by saturated brine (15 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude compound was purified by PREP HPLC to afford Compound **53** (1-(4-(difluoromethoxy)benzyl)-6-isobutyl-8-(2-methylbutyl)hexahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*)-dione) (0.103 g, 40 % yield) as a white solid. MS (ESI) m/z $[M+H]^+$: 452.3. ^{1}H NMR (400 MHz, DMSO d_6) δ 7.42 (d, J = 8.8 Hz, 2 H), 7.14 – 7.24 (m, 3 H), 5.0 – 5.10 (m, 1 H), 4.50 – 4.60 (m, 1H), 3.90 – 4.00 (m, 2 H), 3.60 – 3.70 (m, 1 H), 3.02 – 3.40 (m, 4 H), 2.70 – 2.85 (m, 2 H), 2.0 – 2.10 (m, 1 H), 1.50 – 1.70 (m, 4H), 1.20 – 1.35 (m, 1H), 1.0 – 1.10 (m, 1H), 0.70 – 0.98 (m, 12H).

Biological Examples

Example B1. Broad protection from neurotoxicity by Compound 1a and Compound A19 active metabolite in vitro.

[0309] HGF/MET signaling activation is expected to protect cells, including neurons, from cell death. To demonstrate the HGF/MET augmentation properties of example compounds, the ability of Compound 1a and the active metabolite of Compound A19 to improve viability of cultured primary neurons subjected to chemical insults that produce neuron death through a range of mechanisms was tested. Hydrogen peroxide (H_2O_2) treatment produces toxic oxidative stress. Bacteria-derived lipopolysaccharide (LPS) treatment induces inflammatory cell death. Treatment with excess glutamate (Glut) produces excitotoxicity in neuron cultures. Treatment with 1-methyl-4-phenylpyridinium (MPP^+) causes cell death by inhibition of mitochondrial function.

[0310] Rat primary cortical neuron cultures were grown at 37 °C, 5% CO_2 in Complete Neurobasal Medium supplemented with B27/GDNF/BDNF containing 10% FBS until maturity and were then seeded in 384-well plate at 5,000 cells/well. Cultures were then treated for 15 minutes with 1 uM, 100 nM, 10 nM, 1 nM, or 0.1 nM of Compound 1a. Cells were then subjected to individual conditional insults for 24 hours at the following concentrations: H_2O_2 at 1 uM, LPS at 1 uM, glutamate at 25 uM, or MPP+ at 500 uM. All treatments contained recombinant human HGF (R&D Systems) at a concentration of 5 ng/ml. MK-801 (Dizocilpine)

is a pore blocker of the N-Methyl-D-aspartate (NMDA) receptor, a glutamate receptor, and was used as a positive control.

[0311] To quantify neuroprotection, cell viability was measured with the Cell Titer-Glo Luminescent Cell Viability Assay (Promega, Cat #G7571). Data was normalized to DMSO + HGF control set at 100% cell viability. Statistical analyses for each group (n=4) were performed using one-way ANOVA with Tukey's Multiple comparison test.

[0312] The results are shown in Table 2. Data are reported as statistical significance vs. insult control, with NS = not significant, + = $p<0.05$, ++ = $p<0.01$, and +++ = $p<0.001$.

Table 2. Compound 1a protects rat primary neurons from a variety of neurotoxic insults

	Insult effect (% viability vs vehicle)	[Compound 1a] + HGF (5ng/ml)					Positive Control MK-801 (1 uM)
Insult		1 uM	100 nM	100 nM	1 nM	0.1 nM	
H_2O_2 (1 uM)	64.23	+++	+++	+++	+++	+++	+++
Glutamate (25 uM)	49.55	+++	+++	+++	+++	+++	+++
LPS (1 uM)	49.19	+++	+++	+++	NS	+++	+++
MPP+ (500 uM)	49.85	+++	+++	+++	+++	+++	+++

[0313] In a substantially similar study, the ability of the active metabolite of Compound A19, dihexa, to promote broad neuroprotective effects when challenged by the same range of neurotoxic insults was examined. The Compound A19 active metabolite also demonstrated improvement in cell survival against H_2O_2, Glutamate, LPS, and MPP+ at a range of concentrations as detailed in Table 3.

Table 3. Compound A19 active metabolite protects rat primary neurons from a variety of neurotoxic insults

	Insult effect (% viability vs vehicle)	[Compound A19 active metabolite] + HGF (5ng/ml)				
Insult		1 uM	100 nM	100 nM	1 nM	0.1 nM
H_2O_2 (1 uM)	59.35	+++	+++	+++	+++	+++
Glutamate (25 uM)	54.4	NS	+	+	++	+
LPS (1 uM)	57.1	++	++	+++	++	++
MPP+ (500 uM)	54.3	+++	+++	+++	+++	+++

Data are reported as statistical significance vs. insult control, with NS = not significant, + = $p<0.05$, ++ = $p<0.01$, and +++ = $p<0.001$.

[0314] In this study, Compound 1a and Compound A19 active metabolite treatments acted as an effective neuroprotective treatment at a broad range of concentrations against the entire cohort of insults tested. This result indicates that augmentation of HGF/MET signaling with Compound 1a and Compound A19 active metabolite exerted a potent neuroprotective effect on rat neurons in culture.

Example B2. Neuroprotection against toxic inflammatory challenge

[0315] To assess the neuroprotective effect of compound treatment against inflammatory insult, primary cultured cortical neurons were subjected to lipopolysaccharide (LPS) treatment for 24h along with various compound doses of compounds of Formula (I) or Compound A19. LPS is a potent activator of toll-like receptor 4 (TLR4), which is expressed on both neurons and microglia, and can initiate the release of several pro-inflammatory mediators. For example, LPS induces strong release of tumor necrosis factor-α (TNFα) and IL-6 in primary mouse neuronal cultures, both of which are hallmark pro-inflammatory mediators associated with neurodegeneration and cytotoxicity. To demonstrate the neuroprotective potential of test compounds against inflammation-induced cytotoxicity, a Cell Titer-Glo Luminescent Cell Viability Assay (Promega, Cat #G7571) was utilized. Rat primary cortical neuron cultures were grown at 37 °C, 5% CO_2 in Complete Neurobasal Medium supplemented with B27/GDNF/BDNF containing 10% FBS until maturity. 25 µl of culture volume were seeded in a 384-well plate at 5,000 cells/well, and treated with 1 µM, 100 nM, 10 nM, 1 nM, or 0.1 nM of test compound for 15 minutes. Cells were then subjected to 1 µM LPS challenge for 24 hours. Luminescence measurements to assess relative levels of ATP were performed on an Envision plate reader (Perkin Elmer). Data for DMSO control were set to 100% cell viability, and each group (n=4) were averaged and presented as mean ± SEM.

[0316] Results are shown in Table 4. Compounds 1a, 2a, 5a, 6a, and A19 active metabolite all protected primary neurons from LPS-stimulated inflammatory toxicity at at least one dose tested. Statistical analysis were performed using one-way ANOVA with Tukey's Multiple comparison test. ++ indicates $p < 0.01$ and +++ indicates $p < 0.001$ against DMSO + LPS.

Table 4. Neuroprotection of rat cortical neurons against LPS challenge

Treatment	Dose	Significance of neuroprotection vs 1 uM LPS
A19, active metabolite	1 µM	++
	100 nM	++
	10 nM	+++
	1 nM	++
	0.1 nM	+++
Compound 1a	1 µM	+++
	100 nM	+++
	10 nM	+++
	1 nM	+++
	0.1 nM	+++

Compound 2a	1 μM	+++
	100 nM	+++
	10 nM	+++
	1 nM	+++
	0.1 nM	+++
Compound 5a	1 μM	+++
	100 nM	+++
	10 nM	+++
	1 nM	+++
	0.1 nM	+++
Compound 6a	1 μM	+++
	100 nM	+++
	10 nM	+++
	1 nM	+++
	0.1 nM	+++

Example B3. Neuroprotection and neuronal morphology rescue during neuroinflammatory challenge

[0317] A range of neurodegenerative disease states converge on neuroinflammatory dysfunction related to mitochondrial impairment and the generation of reactive oxygen species (ROS). Chemical disruption of mitochondrial function by treatment of neuron cultures with 1-methyl-4-phenylpyridium (MPP+) or the pesticide rotenone can lead to activation of inflammatory signaling pathways and death via apoptotic and necrotic mechanisms. Similarly, intracellular oxidation of 6-hydroxydopamine (6-OHDA), which is readily absorbed by dopaminergic neurons in culture, leads to production of ROS within the cell, activation of inflammatory signaling, and cell death. In vitro exposure to these neurotoxins has been successfully used to model the effects of neurodegenerative inflammation and identify therapeutic agents that can protect neurons from these effects.

[0318] Midbrain tissue obtained from 15-day old rat (Wistar) embryos was dissected and the ventral mesencephalic region, an area rich in dopaminergic (DA) neurons was removed and dissociated by trypsinization and mechanical passage. Cells were cultured in Neurobasal medium supplemented with B27 (2%), L-glutamine (2 mM), 2% of Penicillin-Streptomycin (PS) solution, 10 ng/mL of Brain-derived neurotrophic factor (BDNF) and 1 ng/mL of Glial-Derived Neurotrophic Factor (GDNF).

[0319] Viable cells were seeded at a density of 40,000 cells/well in 96-well plates and maintained at 37°C in 5% CO_2. On day 6 of culture, Compound A19 active metabolite was added to cultures at a range of concentrations and incubated for 20 minutes. Subsequently,

chemical insults were added to the culture in separate plates as follows: 4 uM of MPP+ for 48 hours, 10 nM of rotenone for 24 hours, and 20 uM of 6-OHDA for 24 hours.

[0320] Following incubation, cell culture supernatant was replaced by a fixative solution of 4% paraformaldehyde in PBS for 20 minutes and prepared for immunostaining. Fixed cultures were stained with a monoclonal anti-Tyrosine Hydroxylase (TH) antibody (1:10000 in PBS, 2 hrs, RT) followed by incubation with appropriate fluorescent secondary antibody and Hoechst dye. Automated fluorescent imaging, 20 images per well, was performed at 10X magnification. Computation image analysis was used to quantify the total number of TH+ neurons, and the total length of the neurite network. Statistical analysis using a one-way ANOVA followed by Fishers LSD test compared inflammatory insult cultures with and without test compound treatment. "NS" indicates that the comparison was not statistically significant, "+" indicates that $p<0.05$.

[0321] The results are shown in Tables 5-7. Treatment of primary neurons with Compound A19 active metabolite reduces the neurotoxic impact of inflammatory-relevant chemical insults by protecting neurons from death ("TH+ neuron number") and promoting the formation of normal, healthy neurite networks ("Neurite Network").

Table 5. Neuroprotection and Neurite Network Length Rescue Following MPP+ Challenge

	MPP+ injury (4 uM, 48hrs)	
A19, Active metabolite Dose (nM)	TH+ neuron number	Neurite Network
0.01	NS	NS
0.1	NS	NS
1	+	NS
10	+	NS
100	+	+
1000	+	+

Table 6. Neuroprotection and Neurite Network Length Rescue Following Rotenone Challenge

	Rotenone injury (10 nM, 24 hrs)	
A19, Active metabolite Dose (nM)	TH+ neuron number	Neurite Network
0.01	NS	NS
0.1	NS	NS
1	NS	NS
10	+	NS
100	+	+
1000	+	+

Table 7. Neuroprotection and Neurite Network Length Rescue Following 6-OHDA Challenge

	6-OHDA injury (20 uM, 24 hrs)	
A19, Active metabolite Dose (nM)	TH+ neuron number	Neurite Network
0.01	NS	NS
0.1	NS	NS
1	+	NS
10	+	+
100	+	+
1000	+	+

Example B4. Rodent Middle Cerebral Artery Occlusion (MCAO) Model of Stroke

[0322] MCAO in animals is induced on Day 0 by cannulating the femoral artery with an intraluminal filament. Occlusion lasts for 90 minutes and reperfusion takes place over 24 hours. Treatment with a test compound (e.g., a compound of Formula (I) or Compound A19) or vehicle is either prophylactic, starting from Day -3 or therapeutic, starting from Day 1 (24 hours post MCAO surgery). On Day 1 (24 hours post MCAO surgery) animals are assessed for modified neurological severity score (MNSS) and behavioral tests of motor function such as grid walking, rotarod, grip strength, and beam walking. On day 8 a second MNSS is recorded. On day 16, MNSS is re-taken and animals are reassessed for motor function. On day 15, animals are sacrificed. Secondary endpoints include staining with 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) to assess infarct volume, histology H&E staining in the caudate putamen and hippocampus, as well as the collection of cerebrospinal fluid and plasma for S-100B, neuron-specific enolase, glial fibrillary acidic protein, myelin basic protein, fatty-acid binding protein, visinin-like protein-1, and inflammatory cytokines tumor necrosis factor, interleukins-1α, 1β, IL-6.

[0323] Animals treated with test compounds in either a prophylactic or therapeutic mode demonstrate improved MNSS scoring and motor function behaviors during the treatment period compared to vehicle-treated animals. At study termination, histological examination reveals a reduction in endpoint markers of stroke severity including reduced markers inflammation, inflammatory cell activation, toxic protein accumulation, and demyelination in compound-treated animals compared to control-treated animals.

Example B5. Rodent Lipopolysaccharide Model of Septic Encephalopathy

[0324] Encephalitis refers to inflammation of the brain that may result in brain swelling fever, headache, confusion, and seizures. The source of neuroinflammation can vary widely, including due to viral, bacterial, parasitic, or fungal infection or due to autoimmune diseases. Lipopolysaccharide (LPS) is a bacterial endotoxin that induces an intense neuroinflammatory

reaction and has been associated with cognitive decline. The ability of Compound A19 to reverse cognitive decline induced by LPS in a mouse model of encephalitis was studied using a T-maze assay. In a separate substantially similar assay, Compounds 1a and 5a were also assessed.

[0325] Spontaneous and continuous alternation is the innate tendency of rodents to alternate free choices in a T-maze (T-CAT) over a series of successive runs. This sequential procedure relies on working memory and is sensitive to various pharmacological manipulations affecting memory processes. The present studies were conducted to evaluate the abilities of Compound A19, Compound 1a and Compound 5a to reverse LPS-induced cognitive deficit in the T-maze assay.

[0326] Compound A19 was dissolved in 0.9% NaCl (saline) and vortexed periodically until complete dissolution and with the pH adjusted between 7.4 and 7.8. Compound A19 was used in concentrations of 0.125, 0.1, 0.05, 0.025 and 0.0125 mg/ml which when given in a volume of 10 ml/kg result in doses of 1.25, 1, 0.5, 0.25 and 0.125 mg/kg, respectively. Compound 1a was dissolved at 160 mg/ml in 100% DMSO and vortexed until complete dissolution. Then the above stock solution was diluted in 20% PEG-400 and 78% 0.9% NaCl, whereby the stock solution comprised 2% of the final solution. Compound 1a was used in concentrations of 0.05, 0.2, 0.8, 1.6 and 3.2 mg/ml which when given in a volume of 10 ml/kg result in doses of 0.5, 2, 8, 16 and 32 mg/kg, respectively. Compound 5a was dissolved at 100 mg/ml in 100% DMSO and vortexed until complete dissolution. Then the above stock solution was diluted in 20% PEG-400 and 78% 0.9% NaCl, whereby the stock solution comprised 2% of the final solution. Compound 5a was used in concentrations of 0.125, 0.25, 0.5, 1 and 2 mg/ml which when given in a volume of 10 ml/kg result in doses of 1.25, 2.5, 5, 10 and 20 mg/kg, respectively.

[0327] Memantine was dissolved in saline. Memantine was used in concentration of 0.1 and 0.01 mg/ml, which when given in a volume of 10 ml/kg result in a dose of 1 and 0.1 mg/kg, respectively. Memantine is approved for symptomatic treatment of Alzheimer's disease was used as a reference compound.

[0328] LPS was dissolved in saline and prepared at a concentration of 0.0125 mg/ml and given intraperitoneally (i.p.) at a dosage volume of 20 ml/kg to yield a dose of 0.25 mg/kg (250 μg/kg).

[0329] Four to five weeks old male CD-1 mice (Janvier; Le Genest St Isle – France) were used for the study.

[0330] Compound A19 was tested at the doses of 1.25, 1, 0.5, 0.25 and 0.125 mg/kg administrated subcutaneously (s.c.) for 14 days with last treatment given 60 min before the T-maze trial. LPS was injected i.p. 2 weeks prior the T-maze trial. Compound 1a was tested at

doses of 0.5, 2, 8, 16, and 32 mg/kg administered orally (p.o.) for 14 days with last treatment given 60 min before the T-maze trial. Compound 5a was tested at the doses of 1.25, 2.5, 5, 10, and 20 mg/kg administered orally (p.o.) for 14 days with last treatment given 60 min before the T-maze trial. LPS was injected i.p. 2 weeks prior the T-maze trial.

[0331] Table 8 shows the administration route and treatment schedule for each treatment group.

Table 8. Treatment Schedule

Group No.	Description	route	Treatment & Time before trial (min)
1	Saline / Vehicle	ip/sc	Once daily from the LPS administration for 14 days. Last treatment 60 min before T-maze
2	LPS / Vehicle	ip/sc	
3	LPS / Memantine 0.1 mg/kg	ip/ip	Once daily from the LPS administration for 14 days. Last treatment 60 min before T-maze
4	LPS / Memantine 1 mg/kg	ip/ip	
5	LPS / A19 1.25 mg/kg	ip/sc	Once daily from the LPS administration for 14 days. Last treatment 60 min before T-maze
6	LPS / A19 1 mg/kg	ip/sc	
7	LPS / A19 0.5 mg/kg	ip/sc	
8	LPS / A19 0.25 mg/kg	ip/sc	
9	LPS / A19 0.125 mg/kg	ip/sc	
10	LPS/Compound 1a 0.5 mg/kg	ip/po	Once daily from the LPS administration for 14 days. Last treatment 60 min before T-maze
11	LPS/Compound 1a 2 mg/kg	ip/po	
12	LPS/Compound 1a 8 mg/kg	ip/po	
13	LPS/Compound 1a 16 mg/kg	ip/po	
14	LPS/Compound 1a 32 mg/kg	ip/po	
15	LPS/Compound 5a 1.25 mg/kg	ip/po	Once daily from the LPS administration for 14 days. Last treatment 60 min before T-maze
16	LPS/Compound 5a 2.5 mg/kg	ip/po	
17	LPS/Compound 5a 5 mg/kg	ip/po	
18	LPS/Compound 5a 10 mg/kg	ip/po	
19	LPS/Compound 5a 20 mg/kg	ip/po	

[0332] The T-maze apparatus was made of gray Plexiglas with a main stem (55 cm long x 10 cm wide x 20 cm high) and two arms (30 cm long x 10 cm wide x 20 cm high) positioned at a 90-degree angle relative to the main stem. A start box (15 cm long x 10 cm wide) was separated from the main stem by a guillotine door. Horizontal doors were also provided to close specific arms during the force choice alternation task.

[0333] The experimental protocol consisted of a single session, which started with one "forced-choice" trial, followed by 14 "free-choice" trials. In the "forced-choice" trial, the animal was confined 5 seconds in the start arm and was then released while either the left or right goal arm was blocked by a horizontal door. It negotiated the maze but eventually it entered the open goal arm and returned to the start position. Immediately after the animal returned to the start position, the left or right goal door was opened and the animal was allowed to choose freely

between the left and right goal arm ("free choice trials"). The animal was considered to have entered into an arm when it placed its four paws in the arm. A session was terminated, and the animal was removed from the maze as soon as 14 free-choice trials had been performed or 10 min had elapsed, whichever event occurred first.

[0334] The percentage of alternation (number of alternation / 14*100) over the 14 free-choice trials was determined for each mouse and was used as an index of working memory performance. This percentage was defined as entry in a different arm of the T-maze over successive trials (i.e. left–right–left–right. etc).

[0335] Analysis of variance (ANOVA) was performed on the result data. Fisher's PLSD test was used for pairwise comparisons and p value ≤ 0.05 were considered significant. The drug-induced reversion of LPS-induced memory deficit was calculated by setting the respective response of the saline/vehicle as 100% and that of the group LPS/vehicle as 0% reversion.

[0336] A single injection of 250 μg/kg LPS resulted in a significant reduction of spontaneous alternation of mice (about 24% reduction in the study assessing Compound A19 and 44% reduction in the study assessing Compound 1a and Compound 5a) as compared to the performance of saline-injected mice (LPS-free) when assessed 2 weeks following injection. Indeed, in the study assessing Compound A19 the alternation rate was 45 and 69% for LPS/Vehicle and Saline/Vehicle groups, respectively. In the study assessing Compounds 1a and 5a, the alternation rate was 44% and 70% for the LPS/Vehicle and Saline/Vehicle groups, respectively. The reduction in the alternation rate of LPS-mice suggests a cognitive deficit. Tables 9 and 10 shows the spontaneous alternation and recovery percentage for each treatment group.

[0337] As shown in Table 9, Compound A19 produced a dose-dependent reversion of the LPS-induced deficit in the mouse T-maze alternation test. Indeed, the alternation rate was 61, 61, 64, 60 and 42% for the doses of 1.25, 1, 0.5, 0.25 and 0.125 mg/kg A19, respectively. These alternation rates correspond to 65, 65, 79, 62 and -12% recovery in the cognitive performance of LPS-mice for the doses of 1.25, 1, 0.5, 0.25 and 0.125 mg/kg A19, respectively. The effect of Compound A19 was significant at all doses except at 0.125 mg/kg. Additionally, Compound 1a produced a dose-dependent reversion of the LPS-induced deficit in the mouse T-maze alternation test. The alternation rate was 49, 59, 56, 51, and 56% for 0.5, 2, 8, 16 and 32 mg/kg, respectively. The reversal rate was 17, 56, 47, 28 and 44 % for 0.5, 2, 8, 16 and 32 mg/kg, respectively. Compound 5a was also assessed for reversal of LPS-induced deficits in the mouse T-maze alternation test. The alternation rate was 59, 58, 61, 58, and 60% for 1.25, 2.5, 5, 10, and 20 mg/kg, respectively. The reversal rate was 58, 53, 64, 53 and 61% for 1.25, 2.5, 5, 10 and 20 mg/kg, respectively.

[0338] LPS-mice treated with Memantine (1 and 0.1 mg/kg) showed 54 and 64% alternation rate, respectively (see Table 9). These correspond to 38 and 76% recovery in the cognitive performance of LPS treated mice. The effect of Memantine was significant at both doses.

[0339] In line with literature findings, memantine produced a significant increase in the spontaneous alternation of LPS-treated mice, which suggests an improved cognitive performance in the T-maze assay. While 0.125 mg/kg A19 (the lowest tested dose) was ineffective in reversing LPS-induced cognitive deficit, higher dose levels (up to 1.25 mg/kg) significantly improved the cognitive performance of LPS-mice.

[0340] The results for Compounds 1a and 5a are shown in Table 10. Compound 1a exhibited significant reversal of LPS-induced cognitive deficits at 2 mg/kg, and was trending at 8 and 32 mg/kg. Compound 5a resulted in significant reversal of LPS-induced cognitive deficits at all doses.

Table 9. Spontaneous Alternation and Recovery Percentage

Group Names	**Spontaneous Alternation (%)**			**Recovery (%)**
	Mean	s.e.m.	n	
Vehicle / Saline	69.3	2.1	10	100
Vehicle / LPS 250 µg/kg	45.0	2.1	10	0
A19 1.25 mg/kg / LPS 250 µg/kg	60.7	1.9	10	58
A19 1 mg/kg / LPS 250 µg/kg	60.7	2.4	10	65
A19 0.5 mg/kg / LPS 250 µg/kg	64.3	3.8	10	79
A19 0.25 mg/kg / LPS 250 µg/kg	60.0	3.0	10	62
A19 0.125 mg/kg / LPS 250 µg/kg	42.1	2.5	10	-12
Memantine 1 mg/kg / LPS 250 µg/kg	54.3	3.2	10	38
Memantine 0.1 mg/kg / LPS 250 µg/kg	63.6	4.3	10	76

Table 10. Spontaneous Alternation and Recovery percentage

Group names	Spontaneous alternation (%)			Recovery (%)
	Mean	s.e.m.	n	
Vehicle / Saline	70.0	2.3	10.0	100
Vehicle / LPS 250µg/kg	44.3	3.0	10.0	0
Cmpd 1a 0.5mg/kg / LPS 250µg/kg	48.6	5.8	10.0	17
Cmpd 1a 2mg/kg / LPS 250µg/kg	58.6	2.8	10.0	56
Cmpd 1a 8mg/kg / LPS 250µg/kg	56.4	4.0	10.0	47
Cmpd 1a 16mg/kg / LPS 250µg/kg	51.4	1.8	10.0	28
Cmpd 1a 32mg/kg / LPS 250µg/kg	55.7	2.6	10.0	44
Cmpd 5a 1.25mg/kg / LPS 250µg/kg	59.3	3.0	10.0	58
Cmpd 5a 2.5mg/kg / LPS 250µg/kg	57.9	2.7	10.0	53

Cmpd 5a 5mg/kg / LPS 250μg/kg	60.7	2.7	10.0	64
Cmpd 5a 10mg/kg / LPS 250μg/kg	57.9	2.9	10.0	53
Cmpd 5a 20mg/kg / LPS 250μg/kg	60.0	2.4	10.0	61

[0341] The results indicate that Compound A19, Compound 1a, and Compound 5a reversed the LPS-induced cognitive disruption in the mouse T-maze assay.

Example B6. Rodent Human tau transgenic mouse model of Frontotemporal Dementia (FTD)

[0342] Transgenic rodents that express the P301S mutations in tau protein (specific to frontotemporal dementia) are used to assess efficacy of test compounds (e.g., a compound of Formula (I) or Compound A19). At approximately 4 months of age, animals are assessed for performance on a battery of cognitive tasks. Then, transgenic animals and their age-matched non-transgenic counterparts are treated with a compound of Formula (I) or Compound A19 or vehicle for a period of approximately 8 weeks. Animals are then reassessed for treatment-induced alterations in cognition at approximately 6 months of age. Following the behavioral battery, animals are sacrificed, and cerebrospinal fluid and plasma are collected and assessed for levels of phosphorylated tau protein as well as inflammatory mediators including tumor necrosis factor alpha, glial fibrillary acidic protein, interleukin-1α, interleukin-1β, and IL-6. Brains are collected and will be assessed for expression and localization of markers of tau phosphorylation or conformational change including pTau181, pTau217, CP13, MC1 Alz50, and PHF1. Brains are also assessed for inflammatory mediators as indicated above as well as markers of microglia such as Iba1 and markers of neurons including NeuN. Furthermore, brains are assessed for markers indicating synapse loss including PSD95 and synaptophysin, and expression of glycogen synthase kinase 3-Beta.

[0343] Animals treated with test compounds have improvement in one or more tests of cognitive function during the study compared to vehicle-treated animals. At study termination, measurements of expression of one or more inflammatory mediators are reduced in animals treated with test compounds compared to vehicle-treated animals. Markers of pathological protein accumulation are reduced in animals treated with test compounds, and markers of synaptic health are improved in animals treated with test compounds compared to animals treated with vehicle.

Example B7. Reduction of pro-inflammatory cytokine expression in macrophage-like cell cultures.

[0344] Neuroinflammation plays a crucial role in disease progression and is a complex phenomenon involving various cells that affect many extra- and intracellular signaling pathways and cytokine production. The macrophage of the brain (microglia) is the main agent of innate

immunity in the CNS and performs various functions, including neuroprotection, in response to inflammatory signals. Activated microglia participate in developing neuroinflammation, responding to toxic exogenous substances (like LPS) or endogenous substances (like amyloid-beta). Here, THP-1 cells were used as an in-vitro cell model to evaluate mechanisms relevant to CNS inflammation. Differentiation with phorbol-12-myristate-13-acetate (PMA) causes THP-1 monocyte cells to acquire functional and morphological resemblance to macrophages. LPS interacts with THP-1 differentiated macrophage through the toll-like receptor 4, triggering the inflammatory response and stimulating pro-inflammatory cytokine release and eventually leading to cellular death. Test compounds were investigated for whether they have anti-inflammatory effects on LPS-challenged macrophage cultures. THP-1-differentiated macrophages were treated with test compounds for 20 minutes, followed by 24 hrs of LPS challenge. Culture supernatants were then collected to analyzed for the presence of pro-inflammatory cytokines: interleukin 1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α).

[0345] Cytokine quantification in cell culture supernatants was accomplished by homogeneous time resolved fluorescence (HTRF) kits to assess levels of IL-1β (Human IL-1β kit, #62HIL1BPEG, Cisbio) and IL-6 (Human IL-6 kit, 62HIL06PET, Cisbio), and by ELISA (Human TNF-α ELISA kit, KHC3011, ThermoFisher) to determine the levels of TNF-α.

[0346] Data analysis was performed via 1-way ANOVA with Tukey post-test compared to LPS treatment cultures using Prism statistical software (GraphPad). Tabular data indicates significance of reduction in culture supernatants for the indicated analyte at the indicated dose.

[0347] The results are collected in Table 11. Treatment with Compounds 1a, 5a, 6a, and A19 active metabolite all significantly reduced the expression of IL-1β and TNF-α at at least one tested dose. Treatment with Compounds 1a, 5a, and A19 active metabolite all significantly reduced the expression of IL-6 at at least one tested dose "NS" indicates a non-significant reduction in the indicated cytokine. "+" indicates a p value < 0.05 in Tukey post test. "++" indicates a p value < 0.01 in Tukey post test.

Table 11: Significant reduction in pro-inflammatory cytokine expression by THP-1 immune cells in vitro

Compound 1a			
Dose	Inflammatory Analyte		
(nM)	IL-1β	IL-6	TNF-α
0.1	++	NS	++
10	++	++	++
1000	++	+	++

Compound 5a			
Dose	Inflammatory Analyte		
(nM)	IL-1β	IL-6	TNF-α
0.1	++	NS	NS
10	++	+	NS
1000	++	++	+

Compound 6a			
Dose	Inflammatory Analyte		
(nM)	IL-1β	IL-6	TNF-α
0.1	NS	NS	NS
10	NS	NS	NS
1000	+	NS	+

Compound A19, active metabolite			
Dose	Inflammatory Analyte		
(nM)	IL-1β	IL-6	TNF-α
0.1	NS	NS	NS
10	++	+	+
1000	+	++	++

Example B8. Reduction in markers of inflammation and neurodegeneration in a TPD43 mouse model by Compounds A19, 5a and 6a

[0348] Extranuclear accumulation of TDP-43 protein is a pathological hallmark of a number of neurological diseases. Mice with transgenic expression of a mutant form of TDP-43 in neurons have become a valuable model recapitulating a number of disease features, including a marked increase in neuroinflammatory-driven cytokine expression. In this study, the ability of test compounds to reduce the neuroinflammatory effects of mutant TDP-43 expression was examined by quantification of inflammatory cytokines IL-6 and TNF-α in the plasma of transgenic TDP-43 mice. Male Prp-TDP43^{A315T} mice (N=10 mice per group) at 3 weeks of age were administered daily treatments of test compounds. Compound A19 was administered via subcutaneous injection, and Compound 5a and Compound 6a were administered via oral gavage. After 2 months of treatment, plasma samples were collected for cytokine quantification. In all cases, cytokine levels detected in untreated transgenic animals were significantly increased over wild type control animals. Plasma IL-6 and TNF-α cytokine expression levels were determined using the ELISA method. The submandibular vein was punctured with a lancet and a blood drop was collected directly on a microtube containing EDTA as anticoagulant. Samples were centrifuged for 15 minutes at 1000 x g at 4°C within 20 minutes of collection. 20 µl of plasma supernatant was stored at -20°C before ELISA analysis.

[0349] Plasma was diluted at 1:10 in sterile PBS, and IL-6 and TNF-α quantification was performed in duplicate for each animal using 10 µl of sample per well in 96-well plates using the ELISA method (IL-6: Signa-Aldrich Cat#RAB0308, TNF-α: Sigma-Aldrich Cat#RAB0477). Statistical analysis was carried out by 2-way ANOVA with Dunnett's multiple comparisons post-test with compound-treated samples compared to vehicle-treated samples.

[0350] The results are shown in Table 12. In this study, Compound A19 significantly reduced IL-6 expression at doses of 0.005, 0.01, and 0.03 mg/kg and significantly reduced TNF-α expression at 0.005 and 0.01 mg/kg. Compound 5a significantly reduced both cytokine levels at doses of 2, 10, and 20 mg/kg. Compound 6a significantly reduced expression of both cytokines at doses of 10 and 20 mg/kg.

Table 12. Compound A19, Compound 5a, and Compound 6a reduce the expression of inflammatory cytokines in a transgenic neurodegenerative model

			Significant decrease in plasma cytokine expression after 2 months of treatment	
Compound	Dose	Route	IL-6	TNF-α
A19	0.005 mg/kg	SC	++++	++++
	0.01 mg/kg	SC	+++++	+
	0.03 mg/kg	SC	+	NS
5a	2 mg/kg	PO	++++	++++
	10 mg/kg	PO	++++	++++
	20 mg/kg	PO	++++	++++
6a	10 mg/kg	PO	++++	++++
	20 mg/kg	PO	++++	++++

Significance of cytokine expression changes in Prp-TDP43^{A315T} mice treated with the indicated compound at the indicated dose after 2 months of treatment compared to vehicle-treated Prp-TDP43^{A315T} animals. SC = subcutaneous injection, PO = oral gavage. Statistical analysis: 2-way ANOVA with Dunnett's multiple comparisons post-test compared to vehicle-treated animals. NS = not significant, + = $p < 0.05$, ++++ = $p < 0.0001$.

Example B9. Reduction of LPS-mediated inflammation in microglial cell cultures.

[0351] Microglia are the resident immune cells of the brain and, when activated by pathological conditions, promote broad-spectrum neuroinflammation. When activated by toxic exogenous substances, microglia produce excessive reactive oxygen species (ROS), nitric oxide (NO), and proinflammatory cytokines, which further propagates disease pathogenesis. BV2 microglia were used as an in-vitro cell model to evaluate mechanisms relevant to inflammation in the CNS. Upon activation of TLR4 with LPS, microglia are activated and trigger excessive oxidative stress, a process that is partially mediated by AKT phosphorylation (pAKT) and activation. Microglia activation also leads to a range of changes in gene expression.

[0352] Compound A19 active metabolite (Compound A19-AM) was investigated for whether it could reduce the inflammatory effect of LPS in microglia. Inflammatory activation of BV2 microglia cells was stimulated by pre-treatment with LPS for 45 minutes, followed by the addition of test compound for 1 hour (pAKT) or 23.5 hours (NO and gene expression panel). For pAKT measurement, cells were lysed and the pAKT measurement was conducted using the HTRF system. For NO production, culture supernatants were collected and assessed for the presence of nitrite, a primary metabolic product of nitric oxide.

[0353] Quantification of nitrite production in cell culture supernatants was accomplished using the Griess Reagent System (Promega, G2930). Measurement of pAKT was assessed

utilizing Phospho-AKT 1/2/3 (Ser473) LANCE Ultra TR-FRET Cellular Detection Kit (CisBio, 64AKSPEH).

[0354] Data analysis was performed via One-way ANOVA with Dunnett's multiple comparisons test compared to LPS-stimulated, vehicle treated cultures using Prism statistical software (GraphPad). Tabular data indicates significance of reduction in the indicated analyte at the indicated dose.

[0355] The results are shown in Table 13. Treatment with Compound A19 active metabolite significantly reduced both AKT phosphorylation and the production of nitrite.

Table 13: Significant reduction in LPS-mediated inflammation in BV2 Microglia

Compound A19 active metabolite		
Dose	Inflammatory Readout	
(nM)	Nitrite	pAKT
0.001	NT	++
0.01	++	NT
1	+++	++
10	NS	NT

"NS" indicates a non-significant reduction NO production. "++" indicates a p value < 0.005 in Dunnett's multiple comparison. "+++" indicates a p value < 0.0005 in Dunnett's multiple comparison; "NT" means not tested.

[0356] Changes in gene expression in BV2 cells in response to stimulation with LPS and whether or not these gene expression changes are influenced by treatment with Compound A19-AM was also examined. Inflammatory activation of BV2 microglia was stimulated by pre-treatment with LPS for 45 minutes followed by the addition of test compound for 23.5 hours. Cell cultures were then lysed and processed for expression profiling via bead-based transcript quantification using the Quantigene multiplex gene expression assay (ThermoFisher) and a custom array of target transcripts relevant to inflammatory activation. Expression of a major component of the inflammasome (NLRP3), cytokines (TNF-α, IL-1β, and IL-6), and inducible nitric oxide synthase (iNOS) were assessed. Expression data were quantified as fold change compared to unstimulated cell cultures. Statistical analysis consisted of one-way ANOVA with Dunnett's multiple comparisons post-test compared to LPS-stimulated lysates. Gene expression data is represented in Table 14. Expression of all inflammatory transcript targets were induced by treatment with LPS. The expression of inflammasome component NLRP3 was significantly reduced by treatment with 100 nM and 1 μM of compound A19-AM. The expression of TNF- α was reduced by treatment with A19-AM at all tested concentrations. Expression of IL-1β was reduced at treatment concentrations of 100 nM and 1 μM, and expression of IL-6 was reduced at

by treatment with 1 μM A19-AM. Expression of iNOS was significantly reduced by treatment with A19-AM at concentrations of 100 nM and 1 μM. Overall, these results suggest that treatment with Compound A19-AM produced anti-inflammatory effects at least partially by reducing the expression of inflammation-related genes.

Table 14: Gene expression changes in response to Compound A19-AM treatment of LPS-stimulated BV2 microglia cells

	Significant reduction in target transcript abundance compared to LPS-stimulated BV2 lysates				
Compound A19-AM dose	NLRP3	TNF-α	IL-1β	IL-6	iNOS
1 pM	NS	++++	NS	NS	NS
10 pM	NS	++++	NS	NS	NS
100 pM	NS	++++	NS	NS	NS
100 nM	++	++++	+	NS	++
1 μM	+	++++	++	+	++

[0357] "NS" indicates a non-significant reduction indicated gene expression compared to LPS-treated cultures."+" indicates a p value < 0.05, "++" indicates a p value < 0.01, "+++" indicates a p value < 0.001 "+++" indicates a p value < 0.0005 in Dunnett's multiple comparison.

Example B10. Reduction of Microglial Activation in Primary Cultures of Cortical Neurons and Microglia Challenged with Aβ 1-42

[0345] Microglial activation is a hallmark of neurodegenerative diseases such as Alzheimer's Disease (AD) and Parkinson's Disease (PD). For example, microglia are activated in response to accumulating amyloid beta (Aβ) peptides and are thought to contribute to AD pathogenesis, as excessive activation can lead to neuroinflammation and neuronal damage. Thus, compounds that can reduce excessive activation of microglia may represent a promising therapeutic avenue for neuroinflammatory conditions. Compound A19 active metabolite (A19-AM) was investigated for whether it can reduce microglia activation in a primary culture of both cortical neurons and microglia injured with Aβ. Microglia were treated with compound A19-AM (3 different treatment conditions as indicated below), HGF (5 ng/ml), or BDNF (50 ng/ml), and challenged with Aβ1-42 for 72 hours. Cultures were then fixed and immunostained for Iba1, a marker for microglial activation.

[0346] Quantification of microglial activation was accomplished by automated measurement of the area of Iba-1 stain in fluorescent images captured of treated cell cultures.

[0347] Data analysis was performed via One-way ANOVA with Fisher's LSD compared to Aβ-challenged, vehicle-treated cultures using GraphPad Prism software. Tabular data indicates

significant reduction of microglial activation for the indicated analyte at the indicated dose and treatment. The results are shown in Table 15. Treatment with Compound A19-AM significantly reduced microglial activation, as measured by Iba1 area.

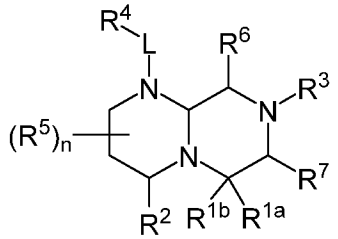
Table 15: Compound A19-AM reduces primary microglia activation in response to Aβ challenge

Treatment	Significant decrease in Iba1 area, compared to vehicle treated (Aβ challenge)
100 nM Compound A19-AM Pre-(60min) and post-(24h) Aβ challenge	NS
1 μM Compound A19-AM Pre-(60min) and post-(24h) Aβ challenge	NS
100 nM Compound A19-AM Pre-(60min) and Co- Aβ challenge	+
1 μM Compound A19-AM Pre-(60min) and Co- Aβ challenge	+
100 nM Compound A19-AM Pre-(60min), Co-, and post-(24h) Aβ challenge	+
1 μM Compound A19-AM Pre-(60min), Co-, and post-(24h) Aβ challenge	+
HGF (5 ng/ml) 15min Pre-Aβ challenge	+
Compound A19-AM 60min pre-Aβ challenge	+

"NS" indicates a non-significant change in Iba1 area. "+" indicates a p value < 0.05 in Fisher's LSD post-test compared to Aβ challenged.

CLAIMS

1. A method of treating a neuroinflammatory condition in a subject in need thereof, comprising administering an effective amount of a compound of Formula (I):



(I)

or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof, wherein:

L is a direct bond, -C(=O)-, -(CR^aR^b)$_m$-C(=O)-, -C(=O)-(CR^aR^b)$_m$-, or -(CR^aR^b)$_m$-;

each R^a and R^b is independently H, C_1-C_6 alkyl, C_2-C_6 alkenyl, or C_2-C_6 alkynyl;

R^{1a} and R^{1b} are independently H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, halo, or C_6-C_{10} arylalkyl;

R^2 is H, oxo, or thioxo;

R^3 is C_2-C_6 alkyl, C_3-C_6 alkenyl, C_3-C_6 alkynyl, C_3-C_{12} cycloalkyl, C_3-C_6 cycloalkylalkyl, C_6-C_{10} arylalkyl, 5- to 10-membered heteroarylalkyl, or 5- to 10-membered heterocyclylalkyl, wherein the 5- to 10-membered heteroarylalkyl or 5- to 10-membered heterocyclylalkyl contains 1-3 heteroatoms selected from nitrogen and oxygen;

R^4 is C_6-C_{10} aryl, 5- to 10-membered heteroaryl, or 5- to 10-membered heterocyclyl, wherein the 5- to 10-membered heteroaryl or 5- to 10-membered heterocyclyl contains 1-3 heteroatoms selected from nitrogen and oxygen;

each R^5 is independently C_1-C_6 alkyl, oxo, or halo;

R^6 is H, C_1-C_6 alkyl, or oxo;

R^7 is H or oxo;

m is 1 or 2; and

n is an integer from 0 to 3;

wherein each C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{12} cycloalkyl, C_3-C_{12} cycloalkylalkyl, C_6-C_{10} aryl, C_6-C_{10} arylalkyl, 5- to 10-membered heteroaryl, 5- to 10-membered heteroarylalkyl, 5- to 10-membered heterocyclyl, and 5- to 10-membered

heterocyclylalkyl is optionally substituted with one to five substituents selected from hydroxyl, halo, amino, C_1-C_6 haloalkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkoxy, cyano, -(C=O)NH_2, nitro, -SO_2(C_1-C_6 alkyl), and -CO_2H.

2. The method of claim 1, wherein L is -C(=O)- or -$(CR^aR^b)_m$-.

3. The method of claim 1 or 2, wherein L is a -C(=O)-.

4. The method of claim 1 or 2, wherein L is -$(CR^aR^b)_m$-.

5. The method of claim 4, wherein R^a and R^b are each H, and m is 1.

6. The method of any one of claims 1-5, wherein R^{1a} and R^{1b} are each independently H; C_1-C_6 alkyl optionally substituted with 1-3 substituents selected from halo, -CO_2H, and -C(=O)NH_2; C_1-C_6 alkoxy; halo; or C_6-C_{10} arylalkyl optionally substituted by 1-3 substituents selected from halo and amino.

7. The method of claim 6, wherein R^{1a} and R^{1b} are each independently H, methyl, fluoro, 2-methylbutyl, -CH_2F, methoxy, -CH_2CO_2H, -$CH_2C(=O)NH_2$, benzyl, or 4-aminobenzyl.

8. The method of claim 6, wherein R^{1a} and R^{1b} are each independently H or C_1-C_3 alkyl.

9. The method of claim 8, wherein R^{1a} is methyl and R^{1b} is H.

10. The method of claim 8, wherein R^{1a} and R^{1b} are each H.

11. The method of any one of claims 1-10, wherein R^2 is H.

12. The method of any one of claims 1-10, wherein R^2 is thioxo.

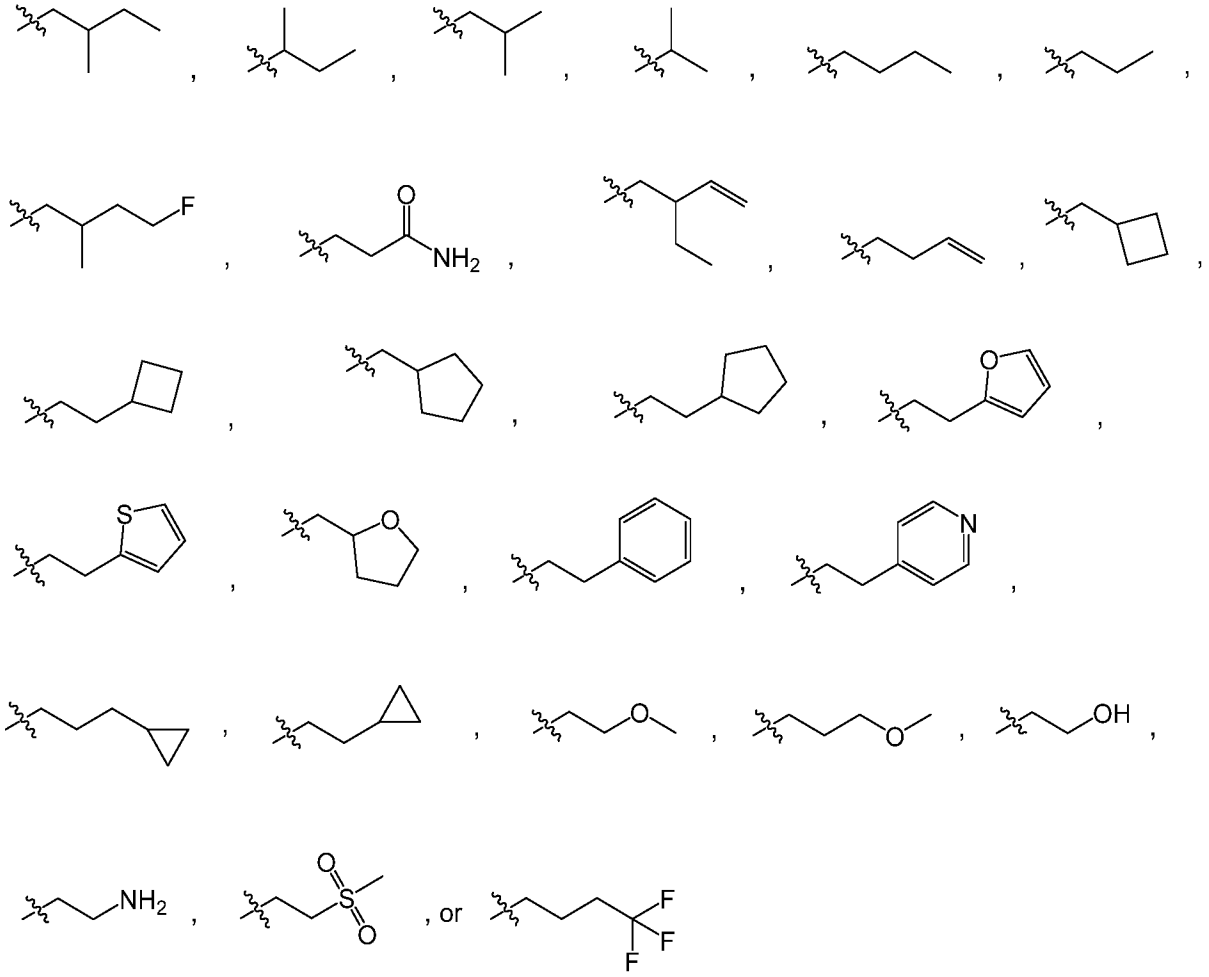
13. The method of any one of claims 1-10, wherein R^2 is oxo.

14. The method of any one of claims 1-13, wherein R^3 is C_3-C_6 alkyl, C_3-C_6 alkenyl, C_3-C_6 alkynyl, C_3-C_{12} cycloalkyl, C_3-C_6 cycloalkylalkyl, C_6-C_{10} arylalkyl, 5- to 10-membered heteroarylalkyl, or 5- to 10-membered heterocyclylalkyl, wherein the alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, arylalkyl, heteroarylalkyl, or heterocyclylalkyl is optionally substituted with one to five substituents selected from hydroxyl, halo, amino, C_1-C_6 haloalkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkoxy, cyano, -(C=O)NH_2, nitro, -SO_2(C_1-C_6 alkyl), and -CO_2H.

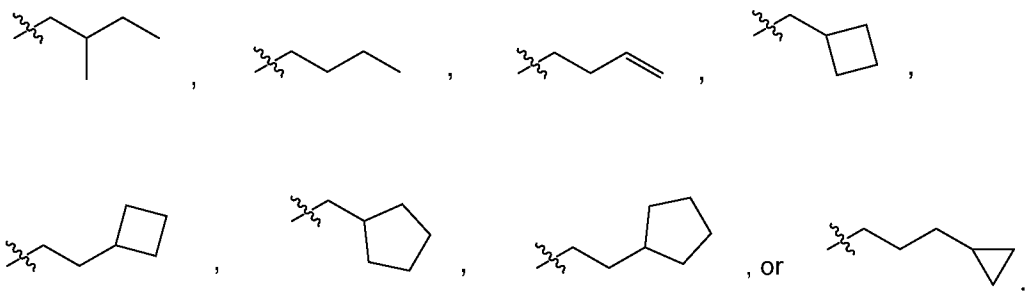
15. The method of any one of claims 1-13, wherein R^3 is C_2-C_6 alkyl optionally substituted by 1-3 substituents selected from halo, C_1-C_3 alkoxy, hydroxy, $-NH_2$, $-SO_2(C_1$-C_3 alkyl), and $-C(=O)NH_2$; C_2-C_6 alkenyl; C_3-C_6 cycloalkylalkyl; 5- to 6-membered heteroarylalkyl; 5- to 6-membered heterocyclylalkyl; or C_6 arylalkyl.

16. The method of claim 15, wherein R^3 is C_2 alkyl substituted by 1-3 substituents selected from C_1-C_3 alkoxy, hydroxy, $-NH_2$, and $-SO_2(C_1$-C_3 alkyl).

17. The method of any one of claims 14-16, wherein R^3 is:



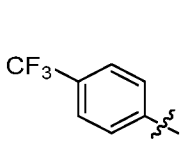
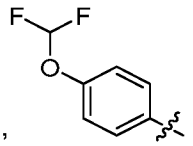
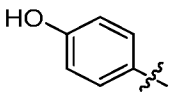
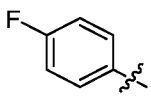
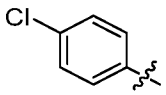
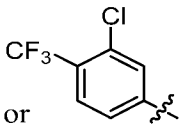
18. The method of claim 17, wherein R^3 is:



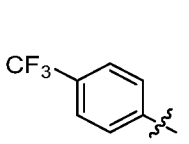
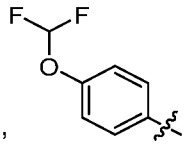
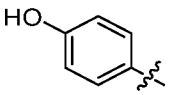
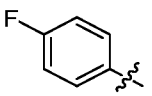
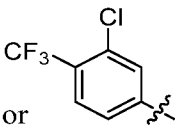
19. The method of any one of claims 1-18, wherein R^4 is C_6-C_{10} aryl optionally substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy.

20. The method of claim 19, wherein R^4 is phenyl substituted with 1-3 substituents selected from $-CF_3$, $-OCHF_2$, -OH, fluoro, and chloro.

21. The method of claim 20, wherein R^4 is:

22. The method of claim 21, wherein R^4 is:

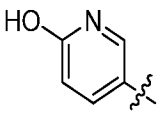
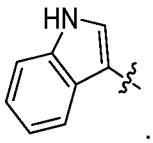






23. The method of any one of claims 1-18, wherein R^4 is 5- to 10-membered heteroaryl optionally substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy.

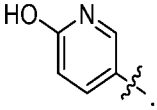
24. The method of claim 23, wherein

R^4 is pyridyl or indolyl optionally substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy.

25. The method of claim 24, wherein

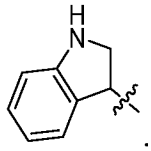
R^4 is  or .

26. The method of claim 25, wherein

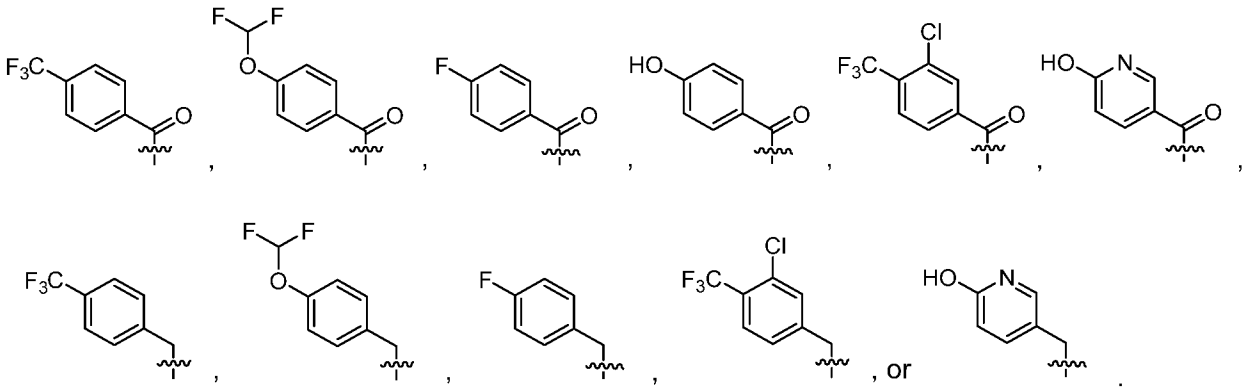
R^4 is .

27. The method of any one of claims 1-18, wherein R^4 is 5- to 10-membered heterocyclyl optionally substituted with 1-3 substituents selected from halo, hydroxyl, C_1-C_6 haloalkyl, and C_1-C_6 haloalkoxy.

28. The method of claim 27, wherein R^4 is indolinyl.

29. The method of claim 28, wherein R^4 is .

30. The method of any one of claims 1-26, wherein -L-R^4 is:



31. The method of any one of claims 1-30, wherein n is 0.

32. The method of any one of claims 1-30, wherein n is 1.

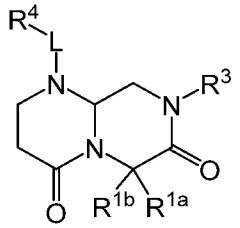
33. The method of claim 32, wherein R^5 is oxo or halo.

34. The method of claim 33, wherein R^5 is oxo or fluoro.

35. The method of any one of claims 1-34, wherein R^6 is H.

36. The method of any one of claims 1-35, wherein R^7 is oxo.

37. The method of any one of claims 1-10, 13-31, 35, and 36, wherein the compound is of Formula (V):



(V) , or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof.

38. The method of claim 37, wherein:

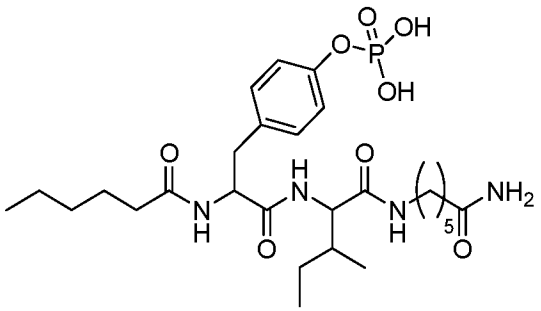
L is -C(=O)- or -CH_2-;

R^{1a} and R^{1b} are independently H or C_1-C_3 alkyl optionally substituted with -CO_2H;

R^3 is C_4-C_5 alkyl, C_4-C_5 alkenyl, or C_1-C_3 alkyl substituted with C_3-C_5 cycloalkyl; and

R^4 is phenyl or pyridyl substituted with 1-3 substituents selected from -CF_3, -$OCHF_2$, -OH, fluoro, and chloro.

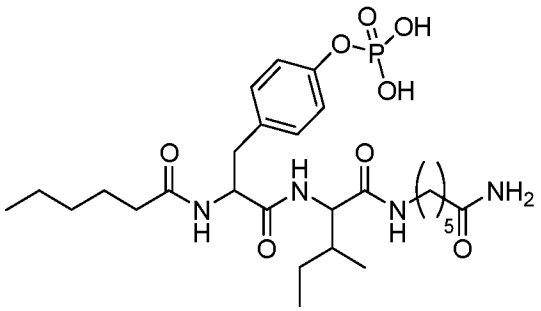
39. A method of treating a neuroinflammatory condition in a subject in need thereof, comprising administering an effective amount of compound A19:



A19

or a pharmaceutically acceptable salt, isotopic form, or stereoisomer thereof.

40. A method of treating a neuroinflammatory condition in a subject in need thereof, comprising administering an effective amount of a compound selected from the compounds of Table 1A and compound A19:



A19

and pharmaceutically acceptable salts, isotopic forms, and stereoisomers thereof.

41. The method of any one of the preceding claims, wherein the neuroinflammatory condition is multiple sclerosis, stroke, a frontotemporal dementia, an encephalopathy, or an encephalitis.

42. The method of any one of claims 1-41, wherein the neuroinflammatory condition is multiple sclerosis.

43. The method of any one of claims 1-41, wherein the neuroinflammatory condition is a stroke.

44. The method of claim 43, wherein the neuroinflammatory condition is an ischemic stroke.

45. The method of claim 43, wherein the neuroinflammatory condition is a hemorrhagic stroke.

46. The method of any one of claims 1-41, wherein the neuroinflammatory condition is a frontotemporal dementia.

47. The method of claim 46, wherein the frontotemporal dementia is idiopathic.

48. The method of claim 46, wherein the frontotemporal dementia is a result of progranulin mutation associated linked to chromosome 17 (p17).

49. The method of any one of claims 1-41, wherein the neuroinflammatory condition is an encephalopathy.

50. The method of claim 49, wherein the encephalopathy is associated with hypoxia, such as small vessel encephalopathy (“Binswanger’s disease”), multi-infarct dementia, asphyxia, or ischemia.

51. The method of claim 49, wherein the encephalopathy is chronic traumatic encephalopathy.

52. The method of any one of claims 1-41, wherein the neuroinflammatory condition is an encephalitis.

53. The method of claim 52, wherein the encephalitis is an autoimmune encephalitis, viral encephalitis or bacterial encephalitis.

54. The method of claim 53, wherein the autoimmune encephalitis is N-methyl D-aspartate receptor (NMDAR)-encephalitis.

55. The method of any one of claims 1-54, wherein the compound reduces inflammation associated with the inflammatory condition.

56. The method of any one of claims 1-55, wherein the compound reduces hypoxia associated with the inflammation.

57. The method of any one of claims 1-56, wherein the compound improves neuronal survival and/or suppresses apoptotic cell death resulting from inflammation.

58. The method of any one of claims 1-57, wherein the compound is formulated as a pharmaceutical composition.

59. The method of any one of claims 1-58, wherein the neuroinflammatory condition is not caused by peripheral inflammation or a disease or disorder of the peripheral nervous system.

60. The method of any one of claims 1-59, wherein the neuroinflammatory condition is not caused by Alzheimer's disease, mild cognitive impairment, Parkinson's disease, Huntington’s disease, amyotrophic lateral sclerosis, spinal cord injury, traumatic brain injury, or sensorineural hearing and vision loss.

61. The method of any one of claims 1-60, wherein the neuroinflammatory condition is not associated with peripheral inflammation or a disease or disorder of the peripheral nervous system.

62. The method of any one of claims 1-61, wherein the neuroinflammatory condition is not associated with Alzheimer's disease, mild cognitive impairment, Parkinson's disease,

Huntington’s disease, amyotrophic lateral sclerosis, spinal cord injury, traumatic brain injury, or sensorineural hearing and vision loss.

63. The method of any one of claims 1-62, wherein the subject is not suffering from Alzheimer's disease, mild cognitive impairment, Parkinson's disease, Huntington’s disease, amyotrophic lateral sclerosis, spinal cord injury, traumatic brain injury, sensorineural hearing and vision loss, or a disease or disorder of the peripheral nervous system.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/020832

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/519 A61P25/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/035587 A1 (MYRIAD GENETICS INC [US]; QABAR MAHER N [US] ET AL.) 29 April 2004 (2004-04-29)	1-39, 41-63
A	page 77, line 21 - page 78, line 1; claims 1-65; tables 2, 3, 4, 5; compounds 1-291	40
X	WO 2017/210489 A1 (M3 BIOTECHNOLOGY INC [US]) 7 December 2017 (2017-12-07) cited in the application claim 20	39
E	WO 2023/114456 A1 (ATHIRA PHARMA INC [US]) 22 June 2023 (2023-06-22) claims 1-42	1-63
X,P	WO 2022/094400 A1 (ATHIRA PHARMA INC [US]) 5 May 2022 (2022-05-05) claims 1-51	1-63

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2023	31/07/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Scheid, Günther

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2023/020832

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004035587 A1	29-04-2004	AU 2003284120 A1	04-05-2004
		CA 2502644 A1	29-04-2004
		US 2005250780 A1	10-11-2005
		WO 2004035587 A1	29-04-2004
WO 2017210489 A1	07-12-2017	AU 2017273857 A1	20-12-2018
		AU 2021266222 A1	02-12-2021
		CA 3026074 A1	07-12-2017
		CN 109563144 A	02-04-2019
		DK 3464336 T3	09-05-2022
		EP 3464336 A1	10-04-2019
		EP 4067368 A1	05-10-2022
		ES 2912035 T3	24-05-2022
		HR P20220479 T1	27-05-2022
		JP 7203943 B2	13-01-2023
		JP 2019525947 A	12-09-2019
		JP 2022051738 A	01-04-2022
		JP 2023036883 A	14-03-2023
		LT 3464336 T	10-05-2022
		PL 3464336 T3	16-05-2022
		RS 63143 B1	31-05-2022
		SI 3464336 T1	30-06-2022
		US 2020010504 A1	09-01-2020
		US 2021340176 A1	04-11-2021
		WO 2017210489 A1	07-12-2017
WO 2023114456 A1	22-06-2023	NONE	
WO 2022094400 A1	05-05-2022	AR 124637 A1	19-04-2023
		AU 2021371026 A1	15-06-2023
		CA 3196582 A1	05-05-2022
		IL 302465 A	01-06-2023
		KR 20230104191 A	07-07-2023
		TW 202233622 A	01-09-2022
		WO 2022094400 A1	05-05-2022